

RNAscope™ Multiomic LS Detection Kit

For use with BOND RX™ System, from Leica Biosystems

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Citing RNAscope in Publications

When describing a procedure for publication using this product, please refer to it as the RNAscope Assay and cite: Wang F, Flanagan J, Su N, Wang L-C, Bui S, Nielson A, Wu X, Vo H-T, Ma X-J and Luo Y. RNAscope: A Novel *In Situ* RNA Analysis Platform for Formalin Fixed Paraffin Embedded Tissues. *J. Mol. Diagnostics*, 2012, 14:22–29.

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Chapter 1. Product Information



Before using this product, read and understand the information in **Appendix L. Safety** of this document.

IMPORTANT! We recommend reading the entire user manual before beginning any protocols.

About this guide

This user manual provides guidelines and protocols to use the RNAscope Multiomic LS Fluorescent Reagent Kit for use with the BOND RX Research Advanced Staining System.

For questions or support, contact your ACD representative at +1 (877) 576-3636.

Product description

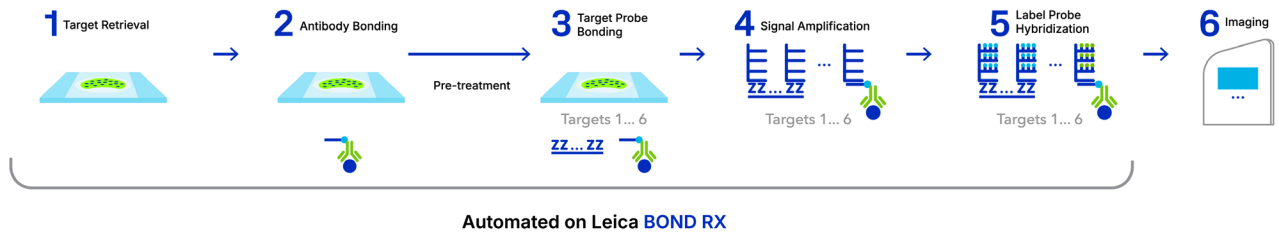
Background

The RNAscope Multiomic LS Assay uses a novel and proprietary method of *in situ* hybridization (ISH) to simultaneously visualize up to six RNA or protein targets in samples mounted on slides. The assay is based on ACD's patented signal amplification and background suppression technology and incorporates signal amplification systems that enable users to investigate expression as well as positional relationship of multiple genes within a cellular context. The RNAscope Multiomic LS Assay allows users to automate the highly sensitive Multiomic Assay using the BOND RX System.

Overview

Figure 1 on page 7 illustrates the RNAscope Multiomic LS Fluorescent Assay procedure, which can be completed on the instrument in ~15 hours. Starting with properly prepared samples, sections are incubated with target retrieval reagents and incubated with antibody to allow binding with their protein targets, then the sections are incubated with pretreatment reagents and RNA-specific probes to hybridize to their target RNAs. The RNAscope Multiomic LS Fluorescent Assay enables up to six independent signal amplification systems each using a different fluorophore to enable independent detection of the six protein or RNA targets or a combination of both. While protein signals are similar to standard fluorescent immune-fluorescence signals, the assay is sensitive enough to detect single RNA transcripts which appear as punctate dots that are visible using a fluorescent microscope or slide scanner.

Figure 1. Procedure overview



| 1. Target retrieval | 2. Antibody binding | 3. Target probe hybridization | 4. Signal amplification and label probe hybridization | 5. Label probe hybridization | 6. Imaging |
|--|------------------------------------|--|--|--|--|
| Start with properly prepared tissue sections and perform target retrieval to allow access to target. | Incubation with target antibodies. | Perform pretreatments and hybridize multiple sets of gene-specific probe pairs to target RNAs. | RNAscope technology sensitively and specifically amplifies signal. | Label probe hybridization and TSA amplification results in deposition of up to six spectrally distinct fluorophores. | Visualize protein and RNA targets using a fluorescent microscope or slide scanner. |

Kit contents and storage

The RNAscope Multiomic LS Assay requires the RNAscope Multiomic LS Probes (for RNA detection), Conjugated antibodies (for protein detection) and the RNAscope Multiomic LS Reagents, available from Advanced Cell Diagnostics.

RNAscope Multiomic LS Probes

The RNAscope Multiomic LS Probes include the user-specified Target Probe and the Positive and Negative Control Probes. Visit <https://acdbio.com/products> to find a gene specific target probe or appropriate control probes. Each target probe contains a mixture of short oligonucleotides designed to bind to a specific target mRNA, and detectable in one of six probe channels C1, C2, C3, C4, C5 or C6. Signal detection is performed using Tyramide Signal Amplification (TSA) linked fluorophores. Different colors are assigned to the C1, C2, C3, C4, C5 and C6 channel tags depending on the TSA Vivid™, Opal™ dye or TSA® plus fluorophore selected for that channel.

Channel C1 target probes are Ready-To-Use (RTU), while channels C2, C3, C4, C5 and C6 probes are shipped as a 50X concentrated stock. To independently detect multiple target RNAs, each target probe must be in a different channel. If you are using only the C2, C3, C4, C5 and C6 probes, you can use Probe Diluent (Cat. No. 300048)

Each probe is sufficient to stain ~30 standard slides. The probes have a shelf life of two years from the manufacturing date when stored as indicated in the following tables:

| Target Probes | | | | | |
|-------------------------------------|--|----------|---|------------------|---------|
| <input checked="" type="checkbox"/> | Reagent | Cat. No. | Content | Quantity | Storage |
| | RNAscope Multiomic LS Target Probe – [species] – [gene] – C1 | Various | Ready-To-Use (RTU) probe for channel C1 | 16 mL x 1 bottle | 2–8°C |
| | RNAscope Multiomic LS Target Probe – [species] – [gene] – C2 | Various | 50X probe for channel C2 | 320 µL x 1 tube | 2–8°C |
| | RNAscope Multiomic LS Target Probe – [species] – [gene] – C3 | Various | 50X probe for channel C3 | 320 µL x 1 tube | 2–8°C |
| | RNAscope Multiomic LS Target Probe – [species] – [gene] – C4 | Various | 50X probe for channel C4 | 320 µL x 1 tube | 2–8°C |
| | RNAscope Multiomic LS Target Probe – [species] – [gene] – C5 | Various | 50X probe for channel C5 | 320 µL x 1 tube | 2–8°C |
| | RNAscope Multiomic LS Target Probe – [species] – [gene] – C6 | Various | 50X probe for channel C6 | 320 µL x 1 tube | 2–8°C |
| Control Probes | | | | | |
| <input checked="" type="checkbox"/> | Reagent | Cat. No. | Content | Quantity | Storage |
| | RNAscope Multiomic LS 6-plex Positive Control Probe-Hs | 323198 | RNAscope Multiomic LS Positive Control Probe for the RNAscope Multiomic LS Fluorescent Assay – <i>POLR2A</i> (C1 channel), <i>PPIB</i> (C2 channel), <i>UBC</i> (C3 channel), <i>HPRT1</i> (C4 channel), <i>ATB</i> (C5 channel), <i>TUBB</i> (C6 channel). | 16 mL x 1 bottle | 2–8°C |
| | RNAscope Multiomic LS 6-plex Negative Control Probe | 323208 | RNAscope Multiomic LS Negative Control Probe for the | 16 mL x 1 bottle | 2–8°C |

| | | | | | |
|--|--|--|--|--|--|
| | | | RNAscope Multiomic LS Fluorescent Assay – <i>dapB</i> (<i>Bacillus subtilis</i> strain, C1-C6 channels). | | |
|--|--|--|--|--|--|

RNAscope Antibodies

RNAscope antibodies are specially conjugated primary or secondary antibodies that are based on RNAscope technology and enable protein detection with enhanced sensitivity. Panels of pre-configured conjugated primary antibodies are composed of sets of antibodies that have been qualified to work either singly or with others of the same panel and can be combined with RNA-specific probes in the same assay for simultaneous detection of protein and RNA targets. Conjugated secondary antibodies are available for pairing with user-supplied primary antibodies to enable detection of additional proteins of interest.

| RNAscope Human Tumor Infiltrating Lymphocyte (TIL) Primary Antibody Panel | | | | | |
|---|-------------------------|----------|-----------|---------------------|---------|
| <input checked="" type="checkbox"/> | Reagent | Dilution | Cat. No. | Quantity | Storage |
| | RNAscope Ab Hs CD4-C3 | 75x | 322949 | 103 uL, (20 slides) | 2–8°C |
| | RNAscope Ab Hs CD8-C4 | 75x | 322951 | 103 uL, (20 slides) | 2–8°C |
| | RNAscope Ab Hs PanCK-C5 | 75x | 322952 | 103 uL, (20 slides) | 2–8°C |
| | RNAscope Ab Hs FoxP3-C6 | 75x | 322953 | 103 uL, (20 slides) | 2–8°C |
| RNAscope Neural Primary Antibody Panel | | | | | |
| <input checked="" type="checkbox"/> | Reagent | Dilution | Cat. No. | Quantity | Storage |
| | RNAscope™ Ab NeuN-C3 | 75x | AB0018-C3 | 105 uL, (20 slides) | 2–8°C |
| | RNAscope™ Ab GFAP-C4 | 75x | AB0028-C4 | 105 uL, (20 slides) | 2–8°C |
| | RNAscope™ Ab IBA-1-C5 | 75x | AB0038-C5 | 105 uL, (20 slides) | 2–8°C |
| RNAscope Secondary Antibodies | | | | | |
| <input checked="" type="checkbox"/> | Reagent | Dilution | Cat. No. | Quantity | Storage |
| | RNAscope anti-rabbit-C1 | 25x | 322954 | 930 uL, (60 slides) | 2–8°C |
| | RNAscope anti-mouse-C2 | 25x | 322956 | 930 uL, (60 slides) | 2–8°C |

RNAscope Multiomic LS Reagents

To perform the RNAscope Multiomic LS assay, the RNAscope Multiomic LS CORE Reagents along with at least one of the Channel Reagents needs to be purchased. The kits provide enough reagents to stain ~ 20 or ~60 standard slides. The assay reagents are then used with RNA-specific probes (if RNA detection is desired), antibodies (if protein detection is desired), TSA linked fluorophores and mounting medium.

The assay reagents are Ready-To-Use (RTU) except for the TSA buffer, and are stored as indicated in the following tables:

| RNAscope Multiomic LS CORE Reagents | | | | |
|-------------------------------------|------------------------------|--|--|---------|
| <input checked="" type="checkbox"/> | Reagent | Quantity 60-slide kit Cat. No. 322930 | Quantity 20-slide kit Cat. No. 323425 | Storage |
| | RNAscope 2.5 LS Protease III | 21 mL x 1 bottle | 8 mL x 1 bottle | 2–8°C |
| | RNAscope Multiomic LS Rinse | 29 mL x 3 bottles | 11 mL x 2 bottles | 2–8°C |
| | RNAscope Multiomic LS AMP 1 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2–8°C |
| | RNAscope Multiomic LS AMP 2 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2–8°C |

| | | | | |
|---|---|---|---|----------------|
| | RNAscope Multiomic LS AMP 3 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope PretreatPro™ | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic LS Hydrogen Peroxide | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic LS DAPI | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic Antibody Diluent | 29 mL x 3 bottles | 14 mL x 3 bottles | 2-8°C |
| RNAscope Multiomic C1 Channel Reagents | | | | |
| <input checked="" type="checkbox"/> | Reagent | Quantity 60-slide kit Cat. No. 322935 | Quantity 20-slide kit Cat. No. 323430 | Storage |
| | RNAscope Multiomic TSA Buffer | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic HRP Blocker | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic LS HRP C1 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| RNAscope Multiomic C2 Channel Reagents | | | | |
| <input checked="" type="checkbox"/> | Reagent | Quantity 60-slide kit Cat. No. 322940 | Quantity 20-slide kit Cat. No. 323435 | Storage |
| | RNAscope Multiomic TSA Buffer | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic HRP Blocker | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic LS HRP C2 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| RNAscope Multiomic C3 Channel Reagents | | | | |
| <input checked="" type="checkbox"/> | Reagent | Quantity 60-slide kit Cat. No. 322945 | Quantity 20-slide kit Cat. No. 323440 | Storage |
| | RNAscope Multiomic TSA Buffer | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic HRP Blocker | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic LS HRP C3 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| RNAscope Multiomic C4 Channel Reagents | | | | |
| <input checked="" type="checkbox"/> | Reagent | Quantity 60-slide kit Cat. No. 322950 | Quantity 20-slide kit Cat. No. 323445 | Storage |
| | RNAscope Multiomic TSA Buffer | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic HRP Blocker | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic LS HRP C4 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| RNAscope Multiomic C5 Channel Reagents | | | | |
| <input checked="" type="checkbox"/> | Reagent | Quantity 60-slide kit Cat. No. 322955 | Quantity 20-slide kit Cat. No. 323450 | Storage |
| | RNAscope Multiomic TSA Buffer | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic HRP Blocker | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| | RNAscope Multiomic LS HRP C5 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |
| RNAscope Multiomic C6 Channel Reagents | | | | |
| <input checked="" type="checkbox"/> | Reagent | Quantity 60-slide kit Cat. No. 322960 | Quantity 20-slide kit Cat. No. 323455 | Storage |
| | RNAscope Multiomic TSA Buffer | 29 mL x 1 bottle | 8 mL x 1 bottle | 2-8°C |

| | | | | |
|--|--------------------------------|------------------|-----------------|-------|
| | RNAscope Multiomic HRP Blocker | 29 mL x 1 bottle | 8 mL x 1 bottle | 2–8°C |
| | RNAscope Multiomic LS HRP C6 | 21 mL x 1 bottle | 8 mL x 1 bottle | 2–8°C |

Required materials and equipment

Assay design

The RNAscope Multiomic LS assay provides flexibility in assay design to enable detection of up to a total of six protein or RNA targets. Proteins can be detected with user-provided primary antibodies paired with ACD’s oligo-conjugated secondary antibodies. These protein targets can be augmented with available pre-qualified conjugated primary antibodies against common human targets. The conjugated secondary antibodies and conjugated primary antibodies are designed to specific RNAscope channels. Probes to RNA targets can be designed to any open channel not used by one of the conjugated antibodies.

Use this table to plan the channels needed for your assay based on your targets.

| Target | RNAscope channel assignment | | | | | |
|-----------------|---|---|---------------------|---------------------|---------------------|---------------------|
| | C1 | C2 | C3 | C4 | C5 | C6 |
| Protein targets | User provided primary antibody-rabbit + RNAscope anti-rabbit-C1 | User provided primary antibody-mouse + RNAscope anti-mouse-C2 | — | — | — | — |
| | — | — | RNAscope Primary C3 | RNAscope Primary C4 | RNAscope Primary C5 | RNAscope Primary C6 |
| RNA targets | RNAscope C1 probe | RNAscope C2 probe | RNAscope C3 probe | RNAscope C4 probe | RNAscope C5 probe | RNAscope C6 probe |

Note: Antibodies are available only in the listed channels. Probes can be designed for any open channel

User-supplied primary antibodies

If you wish to use the RNAscope Multiomic LS assay with your own primary antibodies, these can be paired with the RNAscope conjugated secondary antibodies (listed in the previous tables). This provides the greatest sensitivity and specificity for your selected targets. Your primary antibodies should be hosted in either rabbit (for use with the RNAscope anti-rabbit-C1 antibody) or hosted in mouse (for use with the RNAscope anti-mouse-C2 antibody).

Recommended fluorophores

The RNAscope Multiomic LS assay requires purchase of TSA Vivid from ACD or Opal from Akoya Biosciences. For a 3-plex assay, TSA Vivid dyes are recommended. For a 4 or 6-plex assay, Opal dyes are recommended.

Dilute the fluorophores to the desired working concentration in the TSA Buffer provided in the RNAscope Kit. Choose a dilution factor for each fluorophore based on recommendations from ACD and your specific experimental conditions including target expression levels, tissue quality, or microscope setting. Materials are qualified with 1:1500 dilution for all fluorophores. We cannot guarantee assay results if you use other fluorescent dyes.

Note: To reconstitute dyes, follow the manufacturer instructions available on the tube labels.

Dilute the fluorophores in TSA buffer provided in the Channel Reagent kits.

Below are recommended combinations of fluorophores. Other combinations are also acceptable as long as the spectra are non-overlapping and are compatible with your imaging system while considering tissue autofluorescence.

3-Plex assay using TSA Vivid Fluorophores

| <input checked="" type="checkbox"/> | Fluorophores | Cat. No. | Recommended dilution range |
|-------------------------------------|---------------------------|----------|----------------------------|
| | TSA Vivid Fluorophore 520 | 323271 | 1:750–1:3000 |
| | TSA Vivid Fluorophore 570 | 323272 | 1:750–1:3000 |
| | TSA Vivid Fluorophore 650 | 323273 | 1:750–1:3000 |

Assays using Akoya Biosciences Opal Fluorophores

| <input checked="" type="checkbox"/> | Fluorophores | Akoya Biosciences Cat. No. | Recommended dilution range | 3-plex ² | 4-plex | 5- or 6-plex |
|-------------------------------------|--|----------------------------|----------------------------|---------------------|--------|--------------|
| | Opal 480 Reagent Pack | FP1500001KT | 1:750–1:3000 | | | ✓ |
| | Opal 520 Reagent Pack | FP1487001KT | 1:750–1:3000 | ✓ | ✓ | ✓ |
| | Opal 570 Reagent Pack | FP1488001KT | 1:750–1:3000 | ✓ | ✓ | ✓ |
| | Opal 620 Reagent Pack | FP1495001KT | 1:750–1:3000 | | | ✓ |
| | Opal 690 Reagent Pack | FP1497001KT | 1:750–1:3000 | ✓ | ✓ | ✓ |
| | Opal Polaris 780 Reagent Pack ¹ | FP1501001KT | TSA-DIG: 1:750–1:3000 | | ✓ | ✓ |

| <input checked="" type="checkbox"/> | Fluorophores | Akoya Biosciences Cat. No. | Recommended dilution range | 3-plex ² | 4-plex | 5- or 6-plex |
|-------------------------------------|--------------|----------------------------|-------------------------------|---------------------|--------|--------------|
| | | | Polaris 780: 1:187.5–1:750 | | | |

¹The Opal Polaris 780 Reagent Pack contains two reagents: Opal TSA-DIG and Opal Polaris 780. We recommend diluting Polaris TSA-DIG in TSA buffer, and diluting Opal Polaris 780 in Antibody Diluent/Block from Akoya Biosciences (PN: ARD1001EA). We recommend keeping the dilution factors of Opal TSA-DIG and Opal Polaris 780 at a constant ratio. For example, when using 1:1500 dilution for Opal TSA-DIG, use 1:375 dilution for Opal Polaris 780. When using 1:750 dilution for Opal TSA-DIG, use 1:187.5 dilution for Opal Polaris 780.

²Opal 650 or Polaris 780 may be used instead of Opal 690, depending on your imager configuration.

Required slide scanner or microscope

It is recommended to use a system with multispectral capabilities, especially for imaging tissue with high autofluorescence. For optimal fluorescence detection, we recommend using a high resolution and high sensitivity cooled CCD camera that is 64 μm pixel size or smaller with > 65% peak quantum efficiency. Common models include Orca-Flash 4.0 (Hamamatsu) and Nuance® EX (Perkin Elmer).

| Slide scanner or microscope | Optics |
|--|--|
| <ul style="list-style-type: none"> Akoya PhenolImager HT Leica DM series or equivalent Zeiss Axio Imager, Axioscan or equivalent Inverted microscope if optics and condenser meet requirements. Required excitation/emission filter cube for 6-plex: DAPI/Opal480/Opal520/Opal570/Opal620/Opal690/Opal780 | <ul style="list-style-type: none"> 20X (N.A. 0.75) air 40X (N.A. 0.8) air (recommended) 40X (N.A. 1.3) oil 63X (N.A. 1.3) oil – use for low expression targets, if needed Use 20X and 40X to visualize high expression genes and low expression genes, respectively |

Required materials and equipment from Leica Biosystems

The RNAscope Multiomic LS Fluorescent Assay is designed for the Leica BOND RX and requires specific materials and equipment available *only* from Leica Biosystems.

| <input checked="" type="checkbox"/> | Component | Cat. No. | Storage |
|-------------------------------------|--|----------|---------------------|
| | BOND RX System – automated slide stainer | – | – |
| | BOND 30 mL Open containers | OP309700 | Room temp (20–25°C) |
| | BOND 6 mL Titration containers* | OPT9049 | Room temp (20–25°C) |
| | BOND Research Detection System | DS9455 | Room temp (20–25°C) |
| | BOND Universal Covertile | S21.4611 | Room temp (20–25°C) |
| | BOND Epitope Retrieval Solution 1-1L (RTU) | AR9961 | 2–8°C |
| | BOND Epitope Retrieval Solution 2-1L (RTU) | AR9640 | 2–8°C |
| | BOND Dewax Solution – 1L (RTU) | AR9222 | 2–6°C |
| | BOND Wash Solution 10X Concentrate – 1L | AR9590 | 2–8°C |
| | BOND Aspirating Probe Cleaning System | CS9100 | 2–8°C |
| | BOND Mixing Stations | S21.1971 | Room temp (20–25°C) |

* BOND 7 mL Containers can be used instead but offer less flexibility.

Other user-supplied materials

IMPORTANT! Do not substitute other materials for the SuperFrost® Plus Slides listed in the following table.

| <input checked="" type="checkbox"/> | Description | Supplier | Cat. No. |
|-------------------------------------|--|---------------------------------------|---------------------------|
| | SuperFrost Plus Slides (required) | Fisher Scientific | 12-550-15 |
| | Salmon Sperm DNA, sheared (10mg/ml) | ThermoFisher | AM9680 |
| | Normal Rabbit IgG Control | R&D Systems | MAB1050 |
| | Mouse IgG2A Isotype Control | R&D Systems | MAB003 |
| | ProLong™ Gold Antifade Mountant | Thermo Fisher | P36930; P10144; P36934 |
| | Opal dyes fluorophores (if not using TSA Vivid Dyes from ACD) | Akoya Biosciences | — |
| | Either BOND Primary Antibody Diluent or Antibody Diluent/Block (if Opal Polaris 780 is used) | Leica Biosystems Akoya Biosciences | AR9352 ARD1001EA |
| | Xylene | Fisher Scientific/MLS | X3P-1GAL |
| | 100% alcohol (EtOH) | American Master Tech Scientific/MLS* | ALREACS |
| | 10% neutral-buffered formalin (NBF) | MLS | — |
| | Paraffin wax | MLS | — |
| | 1X PBS | MLS | — |
| | Microtome | MLS | — |
| | Drying oven, capable of holding temperature at 60 +/- 1°C (optional) | MLS | — |
| | Water bath or incubator, capable of holding temperature at 40 +/- 1°C | MLS | — |
| | Tissue-Tek® Vertical 24 Slide Rack | American Master Tech Scientific/MLS | LWSRA24 |
| | Tissue-Tek Staining Dish (4 required) | American Master Tech Scientific/MLS | LWT4457EA |
| | Tissue-Tek Clearing Agent Dish, xylene resistant (2 required) | American Master Tech Scientific/MLS | LWT4456EA |
| | Cover Glass 24 x 50 mm | Fisher Scientific/MLS | 12-545-F |
| | Distilled water | MLS | — |
| | Fume hood | MLS | — |

* Major Laboratory Supplier in North America. For other regions, please check Catalog Numbers with your local lab supplier.

2

Chapter 2. Before You Begin

Prior to running the RNAscope Multiomic LS Fluorescent Assay on your samples for the first time, we recommend that you:

- Become familiar with BOND RX Research Advanced Staining System from Leica Biosystems. Refer to the *BOND RX System User Manual*.
- Run the assay on Control Slides (Cat. No. 310045 for Human HeLa Cell Pellet, and Cat. No. 310023 for Mouse 3T3 Cell Pellet) using the RNAscope Multiomic LS Positive and Negative RNAscope Multiomic Control Probes.

Important procedural guidelines

- Start with properly fixed and prepared sections. Refer to **Chapter 3. Prepare Samples** for preparation of FFPE, fixed-frozen or fresh-frozen slides. For preparation of other sample types, contact support.acd@bio-techne.com.
- Regularly maintain and clean your automated staining instrument.
- Always run positive and negative control probes on your sample to assess sample RNA quality and optimal permeabilization.
- Do not substitute required materials. The assay has been validated with these materials only.
- Follow the protocol exactly for the best results.
- Do not let your sections dry out during the procedure.
- Use good laboratory practices and follow all necessary safety procedures. Refer to **Appendix L. Safety** for more information.

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Chapter 3. Prepare Samples

The following protocols describe formalin-fixed, paraffin-embedded (FFPE), fixed frozen and fresh frozen sample preparation.

IMPORTANT! We highly recommend following these guidelines. We cannot guarantee assay results with other preparation methods.

IMPORTANT! RNA-only staining has been validated by ACD for all sample preparations described in this manual, but only FFPE samples have been fully tested for combined RNA + protein staining. Please see the following guidance for details.

Prepare FFPE sections

Materials required

-
- 10% neutral buffered formalin (NBF)
 - 1X PBS
 - Paraffin wax
 - 95% Ethanol (EtOH)
 - Xylene
 - Microtome
 - Water bath
 - SuperFrost Plus slides
-

Fix the sample

1. Immediately following dissection cut the tissue into blocks of 3–4 mm in thickness.
2. Place the tissue blocks into fixative within **1 HR** of biopsy.
3. Fix the tissue in 10% NBF for **16–32 HRS** at **ROOM TEMPERATURE (RT)**. Fixation time will vary depending on tissue type and size.

 **CAUTION!** Handle biological specimens appropriately.

IMPORTANT! Fixation for <16 HRS or >32 HRS will impair the performance of the assay.

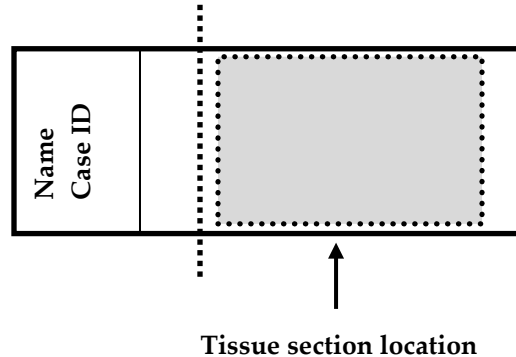
Dehydrate, embed, and cut the sample

IMPORTANT! Use fresh reagents.

1. Wash sample with 1X PBS.
2. Dehydrate sample using a standard ethanol series, followed by xylene.
3. Embed sample in paraffin using standard procedures.

Note: Embedded samples may be stored at room temperature with desiccation. To better preserve RNA quality over a long period (>1 yr), storing at 2–8°C with desiccation is recommended.

- Trim paraffin blocks as needed and cut embedded tissue into 5 +/- 1 µm sections using a microtome.
- Place the paraffin ribbon in a 40–45°C water bath and mount the sections on SUPERFROST® PLUS SLIDES. Place tissue as shown for optimal staining:



IMPORTANT! Do not mount more than one section per slide. Place sections in the center of the slide.

- Air dry slides **OVERNIGHT** at RT.

OPTIONAL STOPPING POINT. Use sectioned tissue within three months. Store sections with desiccants at room temperature.

Prepare fixed-frozen sections

Materials required

-
- 1X PBS
 - 10% Neutral Buffered Formalin (NBF) or 4% Paraformaldehyde (PFA)
 - 100% alcohol (EtOH)
 - Tissue-Tek Vertical 24 Slide Rack
 - Tissue-Tek Staining Dishes
 - Drying oven
-

Fix sample

- If needed, perfuse tissue with freshly prepared 4% Paraformaldehyde (PFA) in 1X PBS, or go directly to step 2.
- Dissect tissue and place in freshly prepared 4% Paraformaldehyde (PFA) for **24 HRS** at 4°C.

Freeze tissue

- Immerse the tissue in 10% sucrose in 1X PBS at 4°C until the tissues sinks to the bottom of the containers (approximately **18 HRS** for brain tissue.)

Note: The time needed for the tissue to sink varies with the tissue type and size.

- Immerse the tissue in 20% sucrose in 1X PBS at 4°C until the tissue sinks to the bottom of the container.
- Immerse the tissue in 30% sucrose in 1X PBS at 4°C until the tissue sinks to the bottom of the container.

4. Freeze the tissue in OCT (Optimal Cutting Temperature) embedding media for TFM (Tissue Freezing Media) with crushed dry ice or iso-pentane or liquid nitrogen.
5. Store tissue blocks in an airtight container at **-80°C**.

Prepare sections

1. Before sectioning, equilibrate the tissue blocks at **-20°C for at least 1 HR in a cryostat**.
2. Section blocks by cutting sections to a thickness of 7 – 15 µm. Mount sections on SuperFrost® Plus slides **ONLY** (other slide types could result in tissue loss).
3. Air dry the slides for **2 HR** at **-20°C** and overnight at **-80°C**. If all slides are not used immediately, store them at **-80°C** for up to **3 MONTHS**.
4. On the day of starting the assay, remove fixed-frozen tissue slides from **-80°C**.
5. Wash the slides with 200 mL 1X PBS for **5 MIN** while moving the rack to remove OCT.
6. Bake slides in drying oven for **15 - 60 MIN** at **60°C**.
7. *Immediately* post-fix slides by immersing them in prechilled 10% NBF or 4% PFA for **15–60 MIN** at **4°C**.

Note: If experiencing issues with sample detachment, the longer post-fix and baking times could be helpful.

Dehydrate and dry the sections

Reagents can be prepared ahead of time. Ensure all containers remain covered.

1. Prepare 200 mL 50% ethanol, 200 mL 70% ethanol, and 2X 200 mL 100% ethanol in Tissue Tek Staining Dishes.
2. Remove the slides from the 10% NBF or 4% PFA, and immerse them in 50% EtOH for **5 MIN** at **RT**.
3. Place the slides in 70% ethanol for **5 MIN** at **RT**.
4. Place the slides in 100% ethanol for **5 MIN** at **RT**.
5. Place slides in fresh 100% ethanol for **5 MIN** at **RT**.
6. Remove slides from ethanol, and let them dry for **5 MIN** at **RT**.

Prepare fresh-frozen sections

Materials required

-
- 1X PBS
 - 10% Neutral Buffered Formalin (NBF) or 4% Paraformaldehyde (PFA)
 - 100% alcohol (EtOH)
 - Tissue-Tek Vertical 24 Slide Rack
 - Tissue-Tek Staining Dishes
-

Section preparation

1. Cryosection the tissue to 10 – 20 µm thickness and place onto SuperFrost Plus slides.
2. Keep the section at **-20°C** to dry for **1 HR**.
3. Store the sections at **-80°C**. Sections can be stored for at least **3 MONTHS** at **-80°C**.

Note: Do not process the slides with any fixative (alcohol or formaldehyde before) this step.

4. The slides can be shipped in dry ice.

Fix the sections

1. Pre-chill 200 ml of 10% NBF or 4% PFA in 1x PBS to 4°C.
2. Remove fresh-frozen tissue slides from -80°C and place in a Tissue Tek Slide Rack.
3. *Immediately* immerse the slides in 200 mL of 10% NBF or freshly prepared 4% PFA.
4. Incubate the slides for at least **90 MIN** at **ROOM TEMPERATURE (RT)**.

Note: Formalin that has been stored for more than six months, exposed to air for more than a week, or used repeatedly may result in suboptimal tissue fixation. 4% PFA must be freshly prepared for each experiment.

Dehydrate the sections

Reagents can be prepared ahead of time. Ensure all containers remain covered.

1. Prepare 200 mL 50% ethanol, 200 mL 70% ethanol, and 2X 200 mL 100% ethanol in Tissue Tek Staining Dishes.
2. Place the slides in 50% ethanol for **5 MIN** at **RT**.
3. Place the slides in 70% ethanol for **5 MIN** at **RT**.
4. Place the slides in 100% ethanol for **5 MIN** at **RT**.
5. Place slides in fresh 100% ethanol for **5 MIN** at **RT**.
Note: If needed, slides can be stored in 100% EtOH at -20°C for up to **1 WEEK**. Prolonged storage may degrade sample RNA.
6. Remove slides from ethanol, and let them dry for **5 MIN** at **RT**.

4

Chapter 4. Determine Pretreatment Conditions

The following protocols describe formalin-fixed, paraffin-embedded (FFPE), fixed-frozen and fresh-frozen sample pretreatment. For other sample types and preparation methods, contact support.acd@biotechne.com for the latest protocols and guidelines.

IMPORTANT! We highly recommend following these guidelines. We cannot guarantee assay results with other preparation methods.

IMPORTANT! RNA-only staining has been validated by ACD for all sample preparations described in this manual, but only FFPE samples have been fully tested for combined RNA and protein staining. Please see the following guidance for details.

Sample preparation for RNA-only staining

Pretreat FFPE sections

Target retrieval

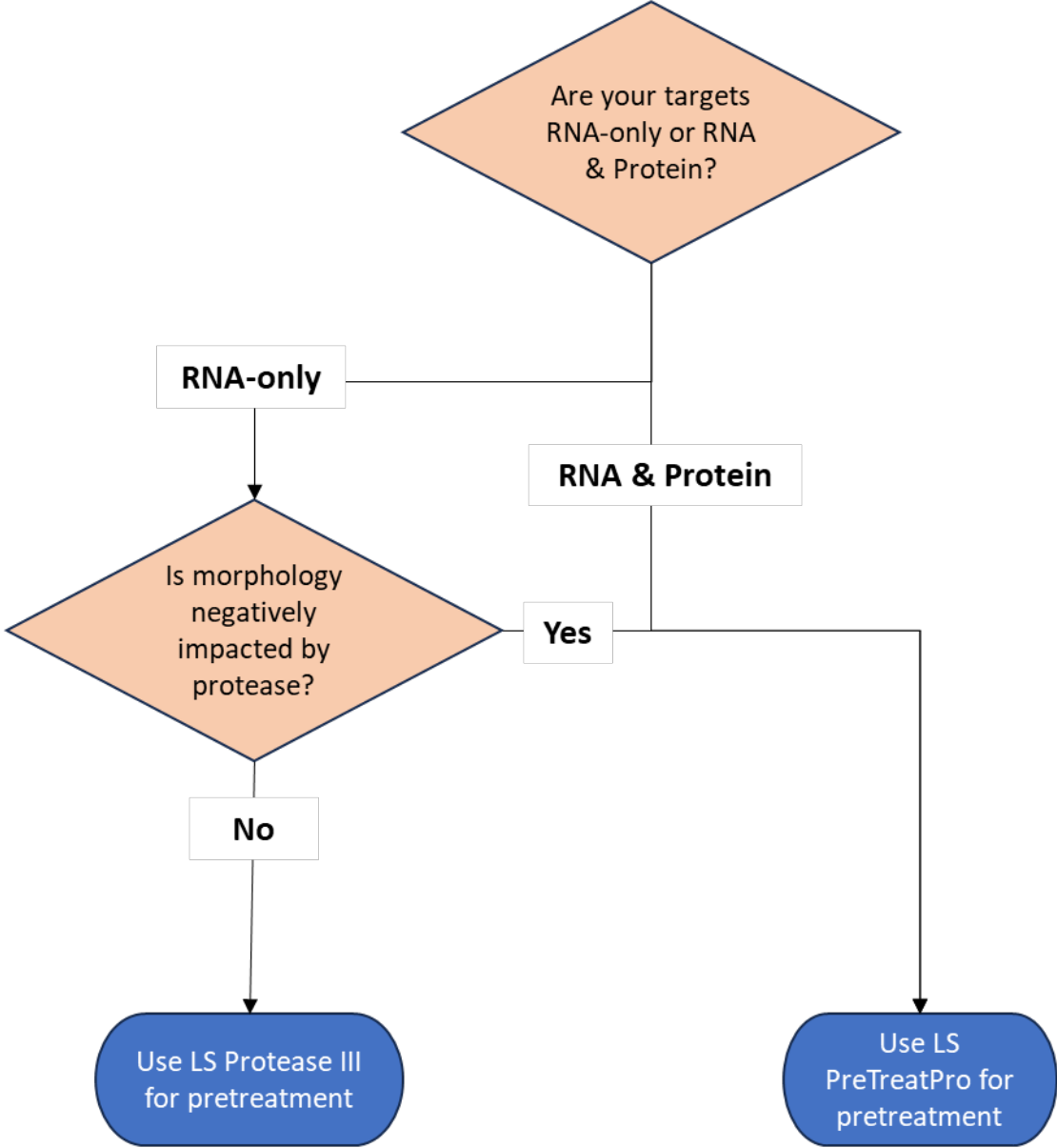
FFPE samples must be de-crosslinked with a target retrieval step. The Multiomic LS Assay uses the BOND RX's ER2 solution exclusively for this step.

Permeabilization

Two options are available:

- Protease-based permeabilization is recommended for experiments that stain only RNA. This option uses LS Protease III.
- Protease-free permeabilization uses the LS PretreatPro reagent which is free of protease enzyme. This allows co-detection of RNA and proteins that were previously incompatible with protease on the same tissue section using immunohistochemistry (IHC). Please refer to **Appendix J** to implement the use of LS PretreatPro reagent on the BOND RX.

To determine the correct permeabilization option, please refer to the following flowchart:



Tissue pretreatment recommendations

Use these conditions as a starting point when tissues are prepared as described in **Chapter 3**. Depending on your tissue type, vary the amount of time for the ER2 and LS Protease III until positive RNA control signal is maximized with minimal/no negative RNA control signal (see **Appendix I**. Pretreatment Guidance for FFPE Samples for a list of tissues)

| Reagent | Mild | Standard |
|---------------------------------|-------------|-------------|
| BOND ER2* | 15 MIN @88C | 15 MIN @95C |
| LS Protease III OR | 15 MIN @40C | 15 MIN @40C |
| LS PretreatPro (see Appendix D) | 30 MIN @40C | |

*Sample types, such as certain xenografts and cell pellets, might require shorter incubation time. For these tissue types, reduce the BOND ER2 incubation time. LS Protease III incubation times can be adjusted but are rarely needed. If you have a tissue type not listed, contact ACD Support at support.acd@bio-techne.com.

Pretreat fixed-frozen sections

Target Retrieval

Fixed-frozen samples must be gently de-crosslinked with a target retrieval step. The RNAscope 2.5 LS Assay uses the BOND RX's ER2 solution exclusively for this step.

Permeabilization

Only LS Protease III has been tested for use with fixed-frozen sections. Check with ACD Support for any updates.

Tissue pretreatment recommendations

Use these conditions as a starting point when tissues are prepared as described in **Chapter 3**. Depending on your tissue type, vary the amount of time for the ER2 and/or LS Protease III until positive control RNA signal is maximized with minimal or no negative RNA control signal (see **Appendix L** for details).

| Reagent | Standard |
|------------------------------|-------------|
| BOND ER2 | 5 MIN @95C* |
| LS Protease III [†] | 15 MIN @40C |

* You might need to create this heat treatment protocol. Please refer to **Appendix F** for further instructions.

† Sample types, such as certain xenografts and cell pellets, might require shorter incubation time. For these tissue types, reduce the BOND ER2 incubation time. LS Protease III time can also be adjusted but is rarely needed.

Pretreat fresh-frozen sections

Target Retrieval

Fresh-frozen sections do not need target retrieval.

Permeabilization

Only LS Protease IV has been tested for use with fresh-frozen sections. Check with ACD Support for any updates.

Tissue pretreatment recommendations

Use this condition as a starting point when tissues are prepared as described in **Chapter 3**. Depending on your tissue type, vary the amount of time for the **Protease IV** until positive RNA control signal is maximized with minimal/no negative RNA control signal (see **Appendix L** for details).

| Reagent | Standard |
|---|------------------|
| LS Protease IV (ACD Part Number 322140) | 30 MIN @Ambient* |

* You might need to create this heat treatment protocol. Please refer to **Appendix F** for further instructions.

Sample preparation for RNA + protein staining

When performing dual staining for RNA and protein targets, detailed guidance is available for FFPE sample types. For other sample types, you can extrapolate from the RNA-only staining guidance provided in the previous section. If you need further assistance, please contact ACD Support.

Pretreat FFPE sections

Target retrieval

FFPE samples must be de-crosslinked with a target retrieval step. The Multiomic LS Assay specifically uses the BOND RX's ER2 solution for this step.

Permeabilization

Only LS PretreatPro has been tested on FFPE samples stained for both RNA and protein. Using LS Protease could negatively impact staining performance of protease sensitive antigens.

Tissue pretreatment recommendations

Use these conditions as a starting point when FFPE tissues are prepared as described in **Chapter 3**. Depending on your tissue type, vary the amount of time for the ER2 until positive RNA control signal is maximized with minimal/no negative RNA control signal (see **Appendix I**. Pretreatment Guidance for FFPE Samples for a list of tissues)

| Reagent | Mild | Standard** |
|-----------------|-------------|-------------|
| BOND ER2 | 15 MIN @88C | 15 MIN @95C |
| LS PretreatPro* | 30 MIN @40C | |

* Sample types, such as certain xenografts and cell pellets, might require shorter incubation time. For these tissue types, reduce the BOND ER2 incubation time. If you have a tissue type not listed, contact ACD Support at support.acd@bio-techne.com.

** Target retrieval for 20min at 100°C can be used if dye trapping or lower antibody signal is observed.

5a

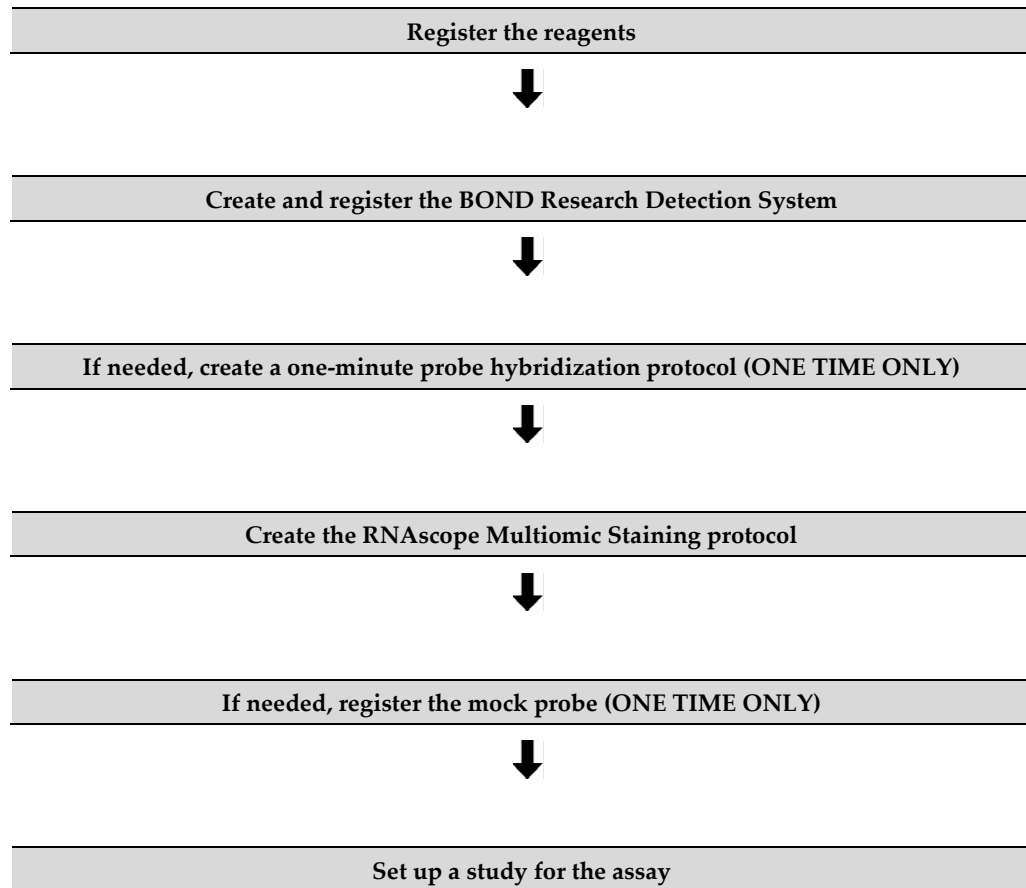
Chapter 5. Set Up a Staining Protocol Using the Mock Probe (software version 6.0 and 7.0)

Use the instructions in this chapter to set up the RNAscope Multiomic LS Assay using the mock probe workaround. This software version is required for runs that combine RNA and protein staining. To set up the assay without the mock probe for RNA-only staining, proceed to **Chapter 6**.

IMPORTANT! We strongly recommend you run the Control Slides (Cat. No. 310045 for human or Cat. No. 310023 for mouse) using the RNAscope Multiomic LS positive and negative control probes along with your samples in every run.

IMPORTANT! Make sure you have software version 6.0 or higher before setting up the assay. The RNAscope Multiomic LS Assay is not supported by earlier software versions.

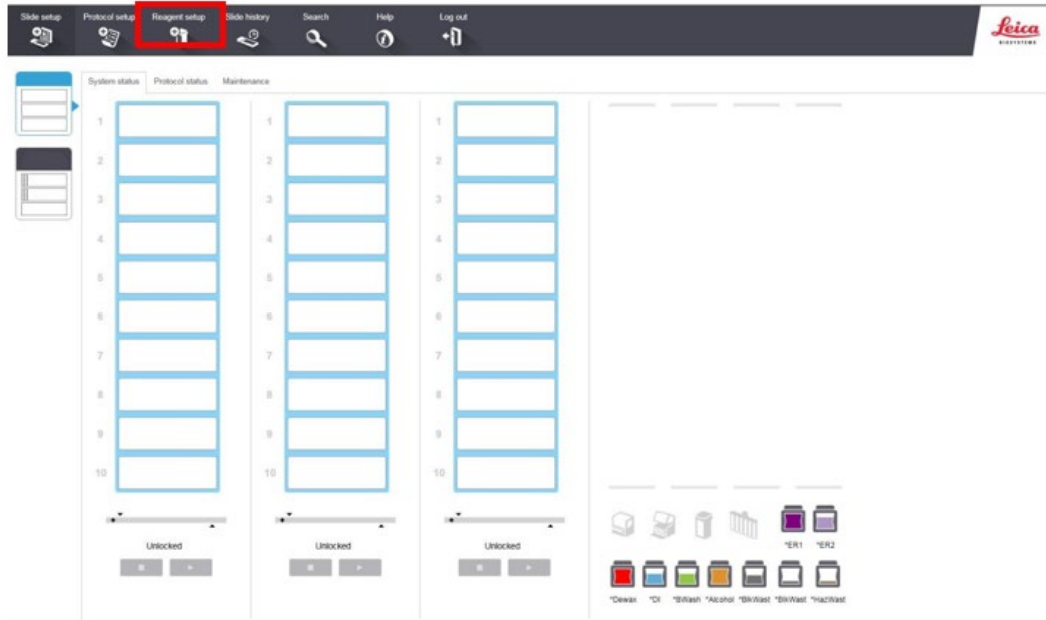
Workflow



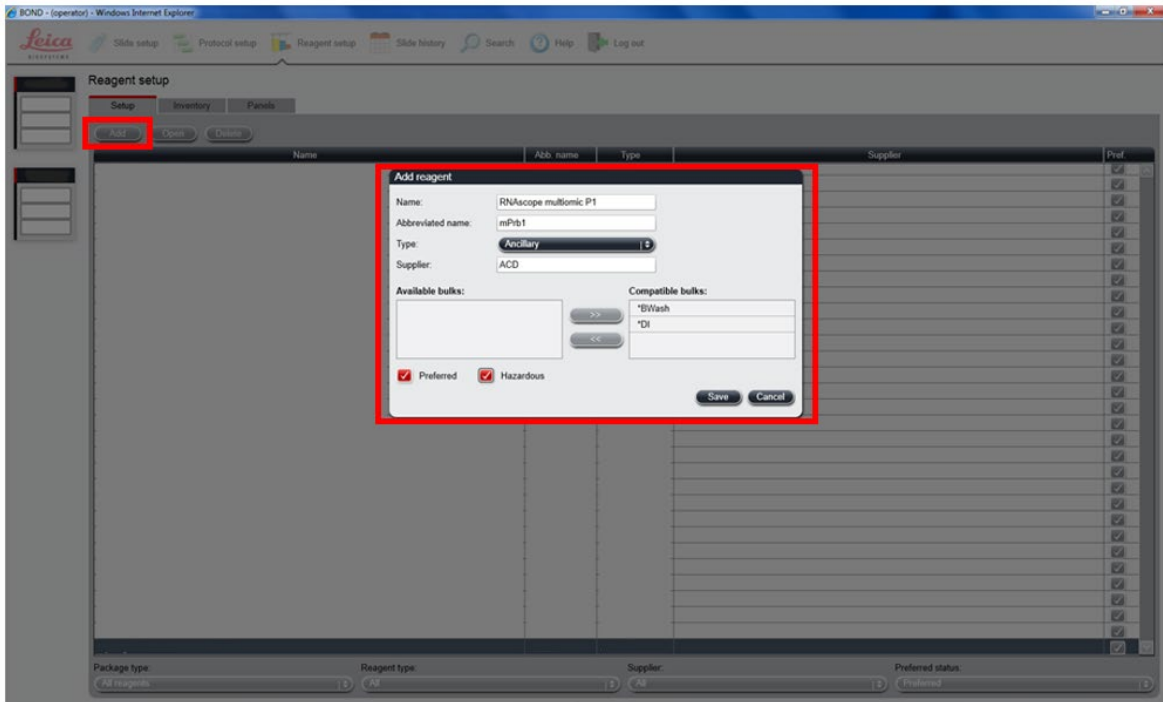
Register the reagents

This step is a “workaround” to the existing 6.0/7.0 software to accommodate the RNAscope Multiomic LS Assay. Your ACD Field Application Specialist (FAS) can help implement this procedure. In summary, a probe is created as an ancillary reagent and added to the staining protocol.

1. Select the **Reagent Setup** icon at the top of the screen.



2. Select **Add** to enter reagent information.



3. Enter a reagent name (for example, RNAscope Multiomic probe 1) in the Name text box.
4. Enter **RNAscope Multiomic P1** (for example) in the Abbreviated name text box.
5. Select **Ancillary** in the Type drop-down menu.
6. Enter **ACD** in the Supplier text box.
7. Check both the **Preferred** and **Hazardous** boxes (for the probes, RNAscope Multiomic Amp 1, and RNAscope Multiomic Amp 3 only).

8. Select **Save**.
9. Repeat Steps 2–7 for each probe and for the rest of the reagents using the container names in the following table. Reagents with an * are pre-installed in the software and do not need to be added:

| Reagents | Container Name | Abbreviated Name | Volume per slide (dead volume not included) | Container Type for 60 slide kit | Container Type for 20 slide kit |
|---|-----------------------------------|------------------|---|---------------------------------|---------------------------------|
| RNAscope Multiomic LS AMP 1 | RNAscope Multiomic LS Amp 1 | MO-Amp1 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS AMP 2 | RNAscope Multiomic LS Amp 2 | MO-Amp2 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS AMP 3 | RNAscope Multiomic LS Amp 3 | MO-Amp3 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS HRP C1 | RNAscope Multiomic LS HRP-C1 | MO-HRPC1 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS HRP C2 | RNAscope Multiomic LS HRP-C2 | MO-HRPC2 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS HRP C3 | RNAscope Multiomic LS HRP-C3 | MO-HRPC3 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS HRP C4 | RNAscope Multiomic LS HRP-C4 | MO-HRPC4 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS HRP C5 | RNAscope Multiomic LS HRP-C5 | MO-HRPC5 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS HRP C6 | RNAscope Multiomic LS HRP-C6 | MO-HRPC6 | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS HRP Blocker | RNAscope Multiomic LS HRP Blocker | HRPBK | 900 µL | 30 mL Open | 30 mL Open |
| Opal/TSA Vivid-fluorophore 1 (user to dilute in TSA buffer) | Multiomic TSA-F1 | MO-TSAF1 | 300 µL | 6 mL Titration | 6 mL Titration |
| Opal/TSA Vivid-fluorophore 2 (user to dilute in TSA buffer) | Multiomic TSA-F2 | MO-TSAF2 | 300 µL | 6 mL Titration | 6 mL Titration |
| Opal/TSA Vivid- | Multiomic TSA-F3 | MO-TSAF3 | 300 µL | 6 mL Titration | 6 mL Titration |

| Reagents | Container Name | Abbreviated Name | Volume per slide (dead volume not included) | Container Type for 60 slide kit | Container Type for 20 slide kit |
|---|---|------------------|---|---------------------------------|---------------------------------|
| fluorophore 3 (user to dilute in TSA buffer) | | | | | |
| Opal-fluorophore 4 (user to dilute in TSA buffer) | Multiomic TSA-F4 | MO-TSAF4 | 300 µL | 6 mL Titration | 6 mL Titration |
| Opal-fluorophore 5 (user to dilute in TSA buffer) | Multiomic TSA-F5 | MO-TSAF5 | 300 µL | 6 mL Titration | 6 mL Titration |
| Opal TSA-DIG † (if Opal 780 is used) | TSA-DIG | TSA-DIG | 300 µL | 6 mL Titration | 6 mL Titration |
| Opal 780† (if Opal 780 is used) | Opal 780 | Opal780 | 300 µL | 6 mL Titration | 6 mL Titration |
| LS RNAscope Multiomic probe mix | (user's choice but must be in container registered as Ancillary in software) | | 300 µL | 6 mL Titration | 6 mL Titration |
| RNAscope Multiomic LS DAPI | *DAPI (Misc) | DAPI_1 | 150 µL | 30 mL Open | 30 mL Open |
| 1X BOND Wash (for Research Detection Kit) | *Detection Wash | Detect Wash | 150 µL | 30 mL Open | 30 mL Open |
| 1X BOND Wash | Mock Probe (registered as Probe RNA in software) | ACDMock | 270 µL | | |
| RNAscope Multiomic LS Hydrogen Peroxide | *Open 0 Haz | | 150 µL | 30 mL Open | 7 mL Titration |
| RNAscope 2.5 LS Protease III | *ACD Enzyme | | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope LS PretreatPro | PretreatPro | PretPro | 300 µL | 30 mL Open | 7 mL Titration |
| RNAscope Multiomic LS Rinse | RNAscope Multiomic LS Rinse | | 600 µL | 30 mL Open | 30 mL Open |

*Indicates this reagent is hard coded in the software by Leica Biosystems.

†These reagents are only needed when Opal Polaris 780 is used in the assay.

Create and register the BOND Research Detection System (one time only)

A BOND Research Detection System from Leica is required to setup the RNAscope Multiomic LS Fluorescent Assay. Your ACD Field Application Specialist (FAS) can help implement this procedure. Each detection system barcode is valid for up to 200 tests of use.

1. Scan the barcode on the tray of a new BOND Research Detection System.
2. To setup a new detection system for the assay, enter **ACD LS Multiplex Detection Kit** in the Name text box.

Note: Creating the detection system needs to be performed only once on each BOND RX controller.

Add research reagent system

Name: ▼

UPI:

Lot N°:

Expiration date:

Reagents

| Pstn. | UPI | Reagent | Vol. (mL) |
|-------|-----|-----------|-----------|
| 1 | | DAPI | |
| 2 | | Bond Wash | |

[Add reagent](#) | [Remove reagent](#)

Add Cancel

3. Place two new BOND 30 mL Open containers on the Research Detection System tray.
4. Scan the first container and select the registration name **DAPI**. You can mix different lots of DAPI in the same container.

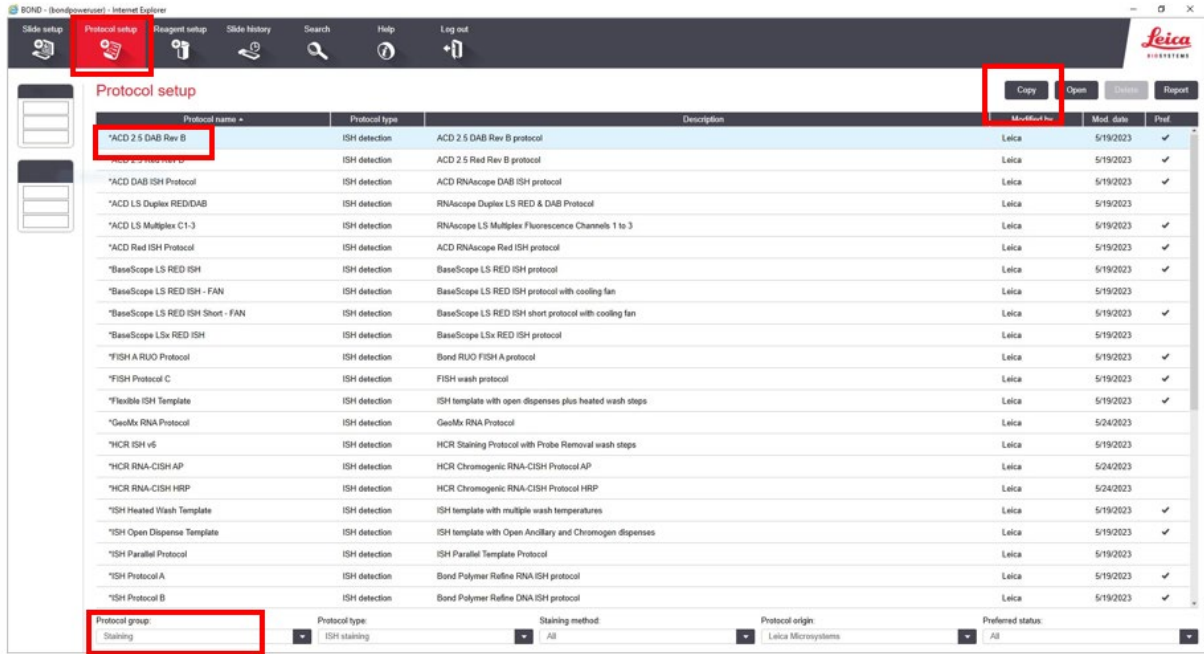
Note: If you prefer not to use DAPI on the instrument or want to perform immunohistochemistry (IHC) steps after the assay, you can use Bond wash in place of DAPI in the staining protocol.

5. Scan the second container and select the registration name **Bond wash**.
6. When one Research Detection System is finished (after 200 tests), register a new detection system by scanning the barcode on the tray and select **ACD LS Multiplex Detection Kit** from the drop-down menu on the right. Enter the new lot number.
7. Register two new 30 mL containers for *DAPI and **Bond wash** by first selecting the reagent name and then scanning the barcode on the container.
8. Select **OK**.

Create a staining protocol

Due to the software workaround for the RNAscope Multiomic LS Fluorescent Assay, unique staining protocols *must be created for each probe*. Your ACD Field Application Specialist (FAS) can help implement this procedure.

1. In the Protocol setup screen, select **Staining** under the Protocol group menu.
2. Highlight the ***ACD 2.5 DAB Rev B** protocol. Select **Copy**.



3. For the following steps, refer to the next figure:
 - a. Change the protocol name for your first probe to **ACD RNAscope Multiomic Protocol P1** in the Name text box, **Multi_P1** in the Abbreviated name text box, and **ACD RNAscope Multiomic Protocol P1** in the Description text box.
 - b. Select **ACD LS Multiplex Detection Kit** from the Preferred detection system menu.
 - c. For Steps 1 –2, change to the desired probe container. In the example, the probe mix is in the **Open 1** container.

Note: The next seven figures display all reagent steps using TSA-DIG and Polaris 780 to label –C6

New protocol properties

Name:

Abbreviated name:

Description:

Staining method: Single Preliminary Final Preferred

BOND RX Import protocol Protocol type: ISH detection

Preferred detection system:

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|---|---------------------------|-------------------------------------|-------------|------------|---------------|
| 1 | | PretreatPro | | <input checked="" type="checkbox"/> | | 0:00 | 150 µL |
| 2 | | PretreatPro | | | 40 | 30:00 | 150 µL |
| 8 | | *RNAscope 2.5 LSx H ₂ O ₂ | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 10:00 | 150 µL |
| 14 | | *Open 1 | User | <input checked="" type="checkbox"/> | | 10:00 | 150 µL |
| 15 | | *Open 1 | User | | 42 | 120:00 | 150 µL |
| 27 | | ACD RNAscope Multiomic Amp 1 | | | 42 | 1:00 | 150 µL |
| 28 | | ACD RNAscope Multiomic Amp 1 | | | 42 | 30:00 | 150 µL |
| 37 | | *LS Rinse | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 5:00 | 150 µL |
| 38 | | *LS Rinse | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 5:00 | 150 µL |

Show wash steps Insert wash | Insert reagent | Delete step

Save Cancel

New protocol properties

Name:

Abbreviated name:

Description:

Staining method: Single Preliminary Final Preferred

BOND RX Import protocol Protocol type: ISH detection

Preferred detection system:

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|------------------------------|---------------------------|-------------------------------------|-------------|------------|---------------|
| 37 | | *LS Rinse | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 5:00 | 150 µL |
| 38 | | *LS Rinse | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 5:00 | 150 µL |
| 43 | | ACD RNAscope Multiomic Amp 2 | | | 42 | 1:00 | 150 µL |
| 44 | | ACD RNAscope Multiomic Amp 2 | | | 42 | 30:00 | 150 µL |
| 53 | | *LS Rinse | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 5:00 | 150 µL |
| 54 | | *LS Rinse | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 5:00 | 150 µL |
| 59 | | ACD RNAscope Multiomic Amp 3 | | | 42 | 1:00 | 150 µL |
| 60 | | ACD RNAscope Multiomic Amp 3 | | | 42 | 15:00 | 150 µL |
| 61 | | ACD RNAscope Multiomic Amp 3 | | | 42 | 1:00 | 150 µL |

Show wash steps Insert wash | Insert reagent | Delete step

Save Cancel

New protocol properties

Name:

Abbreviated name:

Description:

Staining method: Single Preliminary Final Preferred

[Import protocol](#) Protocol type: ISH detection

Preferred detection system:

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|------------------------------------|---------------------------|-------------------------------------|-------------|------------|---------------|
| 60 | | ACD RNAscope Multiomic Amp 3 | | | 42 | 15:00 | 150 µL |
| 69 | | ACD RNAscope Multiomic HRP-C1 | | | 42 | 1:00 | 150 µL |
| 70 | | ACD RNAscope Multiomic HRP-C1 | | | 42 | 15:00 | 150 µL |
| 79 | | *ACD Multiplex TSA-F1 | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 1:00 | 150 µL |
| 80 | | *ACD Multiplex TSA-F1 | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 30:00 | 150 µL |
| 88 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 1:00 | 150 µL |
| 89 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 15:00 | 150 µL |
| 97 | | ACD RNAscope Multiomic HRP-C2 | | | 42 | 1:00 | 150 µL |
| 98 | | ACD RNAscope Multiomic HRP-C2 | | | 42 | 15:00 | 150 µL |

Show wash steps [Insert wash](#) | [Insert reagent](#) | [Delete step](#)

New protocol properties

Name:

Abbreviated name:

Description:

Staining method: Single Preliminary Final Preferred

[Import protocol](#) Protocol type: ISH detection

Preferred detection system:

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|------------------------------------|---------------------------|-------------------------------------|-------------|------------|---------------|
| 98 | | ACD RNAscope Multiomic HRP-C2 | | | 42 | 15:00 | 150 µL |
| 106 | | *ACD Multiplex TSA-F2 | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 1:00 | 150 µL |
| 107 | | *ACD Multiplex TSA-F2 | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 30:00 | 150 µL |
| 115 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 1:00 | 150 µL |
| 116 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 15:00 | 150 µL |
| 124 | | ACD RNAscope Multiomic HRP-C3 | | | 42 | 1:00 | 150 µL |
| 125 | | ACD RNAscope Multiomic HRP-C3 | | | 42 | 15:00 | 150 µL |
| 133 | | *ACD Multiplex TSA-F3 | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 1:00 | 150 µL |
| 134 | | *ACD Multiplex TSA-F3 | Advanced Cell Diagnostics | <input checked="" type="checkbox"/> | | 30:00 | 150 µL |

Show wash steps [Insert wash](#) | [Insert reagent](#) | [Delete step](#)

New protocol properties

Name:

Abbreviated name:

Description:

Staining method: Single Preliminary Final Preferred

[Import protocol](#) Protocol type: ISH detection

Preferred detection system:

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|------------------------------------|----------|---------|-------------|------------|---------------|
| 142 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 1:00 | 150 µL |
| 143 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 15:00 | 150 µL |
| 150 | | ACD RNAscope Multiomic HRP-C4 | | | 42 | 1:00 | 150 µL |
| 151 | | ACD RNAscope Multiomic HRP-C4 | | | 42 | 15:00 | 150 µL |
| 158 | | ACD Multiplex TSA-F4 | | ✓ | | 1:00 | 150 µL |
| 159 | | ACD Multiplex TSA-F4 | | ✓ | | 30:00 | 150 µL |
| 167 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 1:00 | 150 µL |
| 168 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 15:00 | 150 µL |

Show wash steps [Insert wash](#) | [Insert reagent](#) | [Delete step](#)

New protocol properties

Name:

Abbreviated name:

Description:

Staining method: Single Preliminary Final Preferred

[Import protocol](#) Protocol type: ISH detection

Preferred detection system:

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|------------------------------------|----------|---------|-------------|------------|---------------|
| 175 | | ACD RNAscope Multiomic HRP-C5 | | | 42 | 1:00 | 150 µL |
| 176 | | ACD RNAscope Multiomic HRP-C5 | | | 42 | 15:00 | 150 µL |
| 183 | | ACD Multiplex TSA-F5 | | ✓ | | 1:00 | 150 µL |
| 184 | | ACD Multiplex TSA-F5 | | ✓ | | 30:00 | 150 µL |
| 192 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 1:00 | 150 µL |
| 193 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 15:00 | 150 µL |
| 200 | | ACD RNAscope Multiomic HRP-C6 | | | 42 | 1:00 | 150 µL |
| 201 | | ACD RNAscope Multiomic HRP-C6 | | | 42 | 15:00 | 150 µL |

Show wash steps [Insert wash](#) | [Insert reagent](#) | [Delete step](#)

New protocol properties

Name:

Abbreviated name:

Description:

Staining method: Single Preliminary Final Preferred

BOND RX Import protocol Protocol type: ISH detection

Preferred detection system:

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|------------------------------------|----------|---------|-------------|------------|---------------|
| 207 | | ACD RNAscope Multiomic HRP-C6 | | | 42 | 15.00 | 150 µL |
| 201 | | ACD RNAscope Multiomic HRP-C6 | | | 42 | 15.00 | 150 µL |
| 208 | | TSA-DIG | | ✓ | | 1.00 | 150 µL |
| 209 | | TSA-DIG | | ✓ | | 30.00 | 150 µL |
| 215 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 1.00 | 150 µL |
| 216 | | ACD RNAscope Multiomic HRP blocker | | | 42 | 15.00 | 150 µL |
| 222 | | Polaris 780 | | ✓ | | 1.00 | 150 µL |
| 223 | | Polaris 780 | | ✓ | | 30.00 | 150 µL |
| 229 | | *DAPI | Other | ✓ | | 10.00 | 150 µL |

Show wash steps Insert wash | Insert reagent | Delete step

4. Click **Show wash** steps to also view the wash steps. Insert BOND Washes to match each of the protocol steps shown.
5. Compare and confirm the on-screen protocol with the protocol listed in **Appendix A**.
 - a. Refer to Appendices B-E for other 6-plex assay permutations.
6. Select **Preferred** in the top right corner of the window.

New protocol properties

Name:

Abbreviated name:

Description:

Staining method: Single Preliminary Final Preferred

BOND RX Import protocol Protocol type: ISH detection

Preferred detection system:

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|---|---------------------------|---------|-------------|------------|---------------|
| 1 | | PretreatPro | | ✓ | | 0.00 | 150 µL |
| 2 | | PretreatPro | | | 40 | 30.00 | 150 µL |
| 8 | | *RNAscope 2.5 LSx H ₂ O ₂ | Advanced Cell Diagnostics | ✓ | | 10.00 | 150 µL |
| 14 | | *Open 1 | User | ✓ | | 10.00 | 150 µL |
| 15 | | *Open 1 | User | | 42 | 120.00 | 150 µL |
| 27 | | ACD RNAscope Multiomic Amp 1 | | | 42 | 1.00 | 150 µL |
| 28 | | ACD RNAscope Multiomic Amp 1 | | | 42 | 30.00 | 150 µL |
| 37 | | *LS Rinse | Advanced Cell Diagnostics | ✓ | | 5.00 | 150 µL |

Show wash steps Insert wash | Insert reagent | Delete step

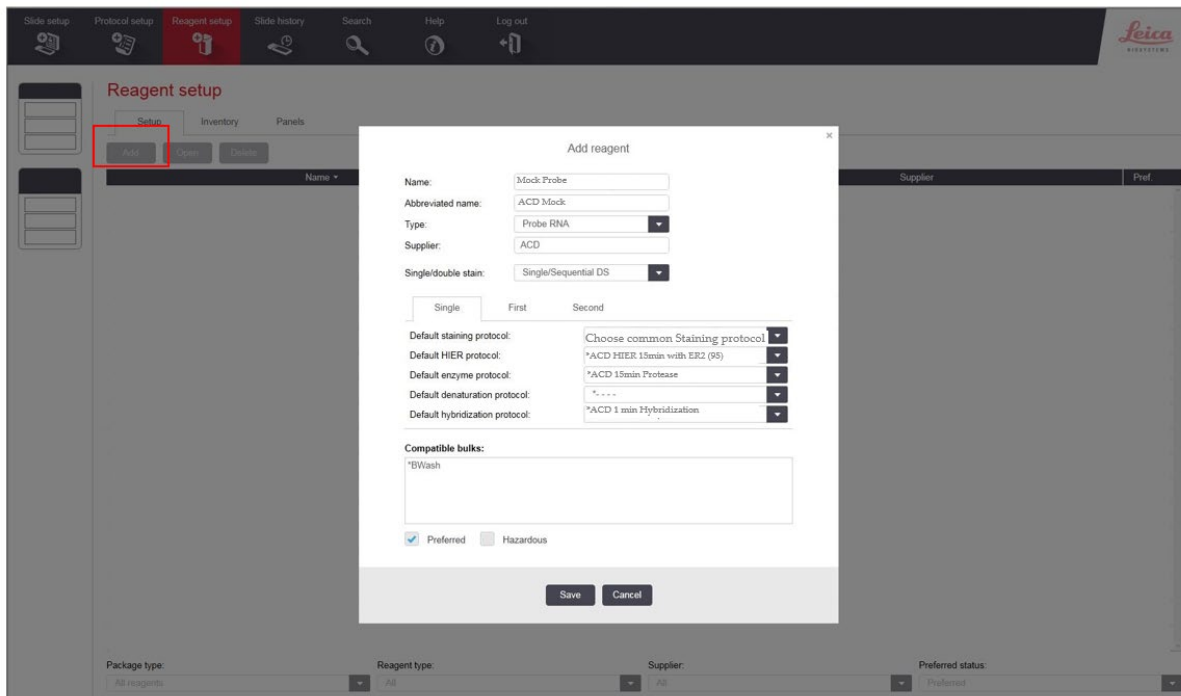
7. Select **Save**.
8. Click **Next** to proceed. Ignore any pop-ups that may appear on the screen.
9. Create a new probe protocol.

Note: You must create a new protocol for each new probe name you use.

Register the mock probe (if not already created)

Create a mock probe in the reagent set up.

1. Click the **Reagent setup** icon.



1.

2. Select **Add**.
3. Enter **Mock Probe** in the Name and **ACD Mock** in Abbreviated name text boxes.
4. Select **Probe RNA** in the Type drop-down menu. Enter **ACD** in the Supplier text box.
5. Select **ACD RNAscope Multiomic Protocol P1** (or your most frequently used protocol) as the Default staining protocol.

6. Select ***ACD HIER 15min with ER2 (95)** as the Default HIER protocol.
7. Select ***ACD 15min Protease** as the Default enzyme protocol.
8. Leave the Default denaturation protocol blank.
9. Select **ACD 1 min Hybridization** as the Default hybridization protocol.
10. Select **Save**.
11. Fill a 30 mL Open container with 1X Bond Wash from the instrument's bulk container and label it **Mock Probe**. This container will be registered as a Mock Probe in the software.

Prepare the instrument reagents

1. Fill the DAPI container with DAPI and the Bond Wash container with Leica Biosystems' 1X Bond Wash. The kit requires 150 µL DAPI or Bond Wash per slide.
2. Carefully transfer all other RNAscope Multiomic LS kit reagents *except for the TSA buffer* into empty 30 mL BOND Open containers.
3. If you haven't already done so, fill a 30 mL Open container with 1X Bond Wash and register this container as **Mock Probe** in the software.

Note: Before each run, make sure you have enough of each reagent. See the table on page 26 for the reagent volume required per slide.

IMPORTANT! Do not introduce bubbles into the solutions by shaking the containers. To mix reagents, gently invert the containers several times. If bubbles are present, leave the containers out at room temperature until the bubbles dissipate.

Note: You may use your own DAPI, or other counterstain, in place of the DAPI provided in the kit.

4. Fill the *Spectral DAPI container with DAPI and the *Bond Wash container with 1X BOND Wash. The kit requires 150 µL DAPI or 1X BOND Wash per slide.
5. Prepare the LS RNAscope Multiomic target probe mix:
 - a. Determine the volume of probe needed (volume needed for the total number of slides plus container dead-volume). If using Mock Probe workaround, you will require 300 µL of RNAscope probe mix per slide plus dead volume for the container. Make sure to add enough dead-volume to your calculation depending on the container type used:
 - 2.5 mL dead-volume when using a BOND 30 mL Open container.
 - 1 mL dead-volume when using a BOND 7 mL Open container.
 - 600 µL dead-volume when using a BOND Titration container (6 mL).
 - b. Dilute the 50X C2 and C3, C4, C5 and C6 probe stocks 1:50 into the Ready-To-Use C1 probe. For example, add 320 µL 50X C2 probe and 320 µL 50X C3 probe to a tube, then add enough C1 probe to bring the final volume to 16 mL.
 - c. Transfer the LS RNAscope Multiomic probe mix into the appropriate Bond container,
 - d. Reminder: probe containers must be registered as **Ancillary** in the software.

Note: The LS RNAscope Multiomic probe mix is stable for one year at 2–8°C.

6. Prepare the Opal fluorophore dilutions:
 - a. Determine the volume of Opal fluorophore needed, and make sure to add dead volume per container (0.6mL for 6 mL Titration container).
 - b. Dilute the Opal fluorophore stock using the TSA buffer provided in the reagent kit.
 - c. Add the diluted fluorophores to the appropriate Leica containers.

- d. For best results, assign brighter fluorophores to low expressors, develop high expressors last and low expressors first, and assign co-expressing markers to spectrally distinct fluorophores.

| Reagents | Recommended dilution range |
|---------------------------------------|---|
| Opal 480* | 1:750–1:3000 (in TSA buffer) |
| Opal 520* | 1:750–1:3000 (in TSA buffer) |
| Opal 570* | 1:750–1:3000 (in TSA buffer) |
| Opal 620* | 1:750 -1:3000 (in TSA buffer) |
| Opal 690* | 1:750–1:3000 (in TSA buffer) |
| Opal TSA-DIG* (if using Opal 780) | 1:750–1:3000 (in TSA buffer) |
| Opal Polaris 780* (if using Opal 780) | 1:187.5–1:750+ (in Akoya or Bond diluent) |

* Reconstitute all Opals (except Opal Polaris 780) with 75 µL Dimethylsulfoxide (DMSO).
Reconstitute Opal Polaris 780 with 300 µL double distilled water (ddH₂O).

† We recommend keeping the dilution factors of Opal TSA-DIG and Opal Polaris 780 at a constant ratio. For example, when using 1:1500 dilution for Opal TSA-DIG, use 1:375 dilution for Opal Polaris 780. When using 1:750 dilution for Opal TSA-DIG, use 1:187.5 dilution for Opal Polaris 780

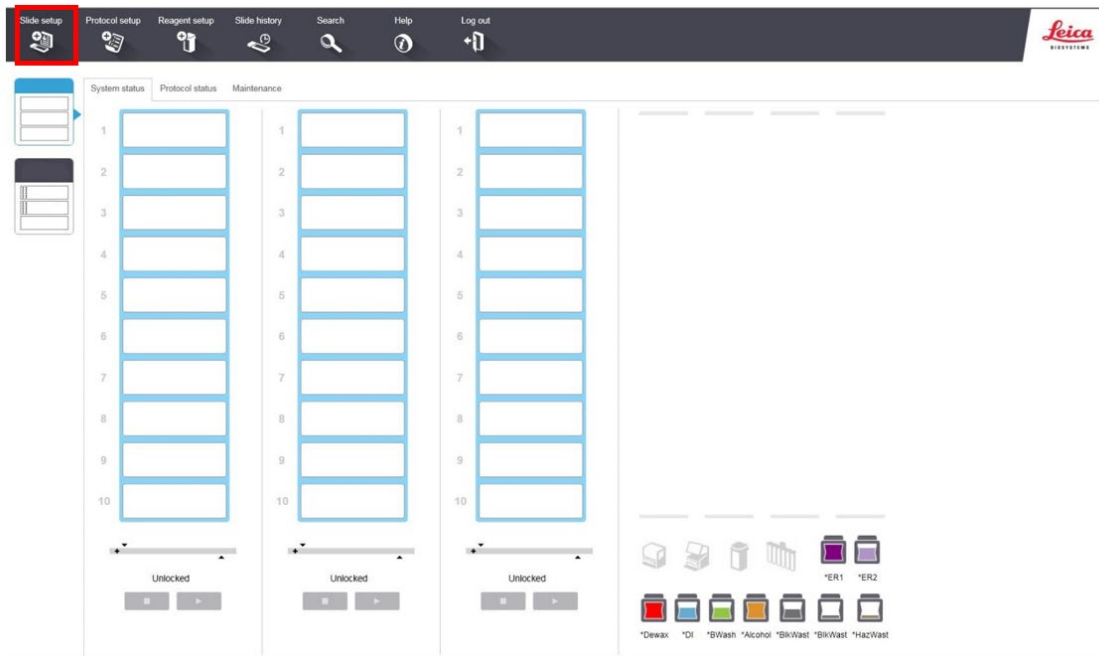
Scan containers into the software

1. Using the Barcode Scanner, scan the barcode located on the front of the BOND Open container. A window appears.
2. From the drop-down menu, select the corresponding name of the reagent as shown in the table on page 26 under **Container name**.

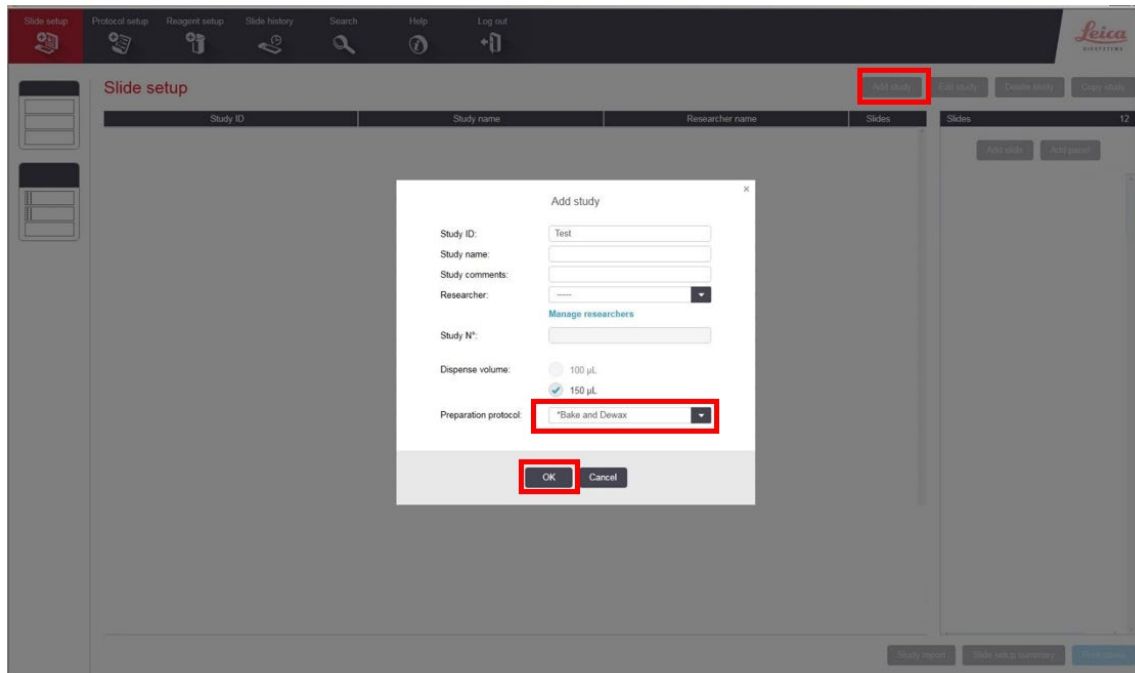
Set up a study for the assay

IMPORTANT! When performing a 6-plex assay, the system can support a maximum of one tray. Follow the protocol steps listed in **Appendices A-E**. Adding any additional steps will prevent the system from running the experiment.

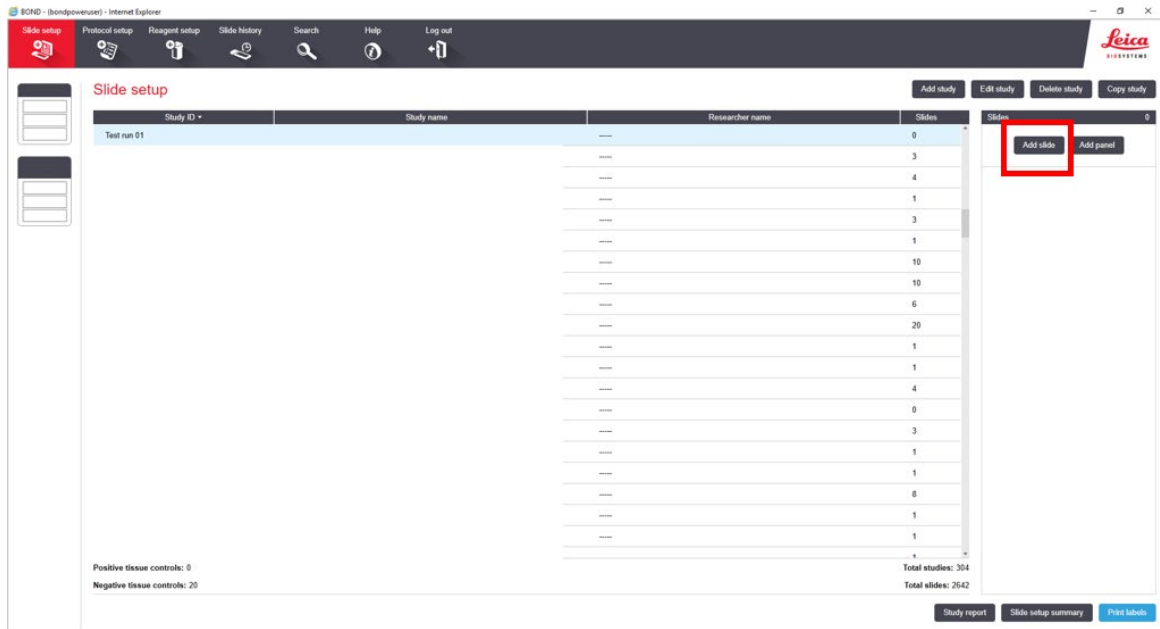
1. To build a study for the RNAscope Multiomic LS Assay, select the **Slide setup** icon at the top of the screen.



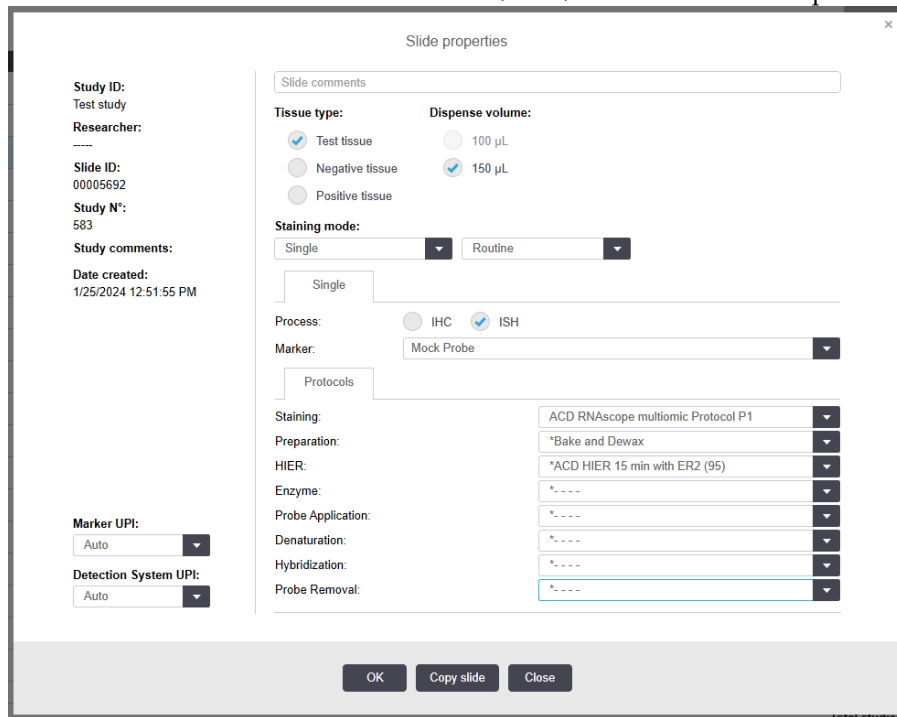
2. Select **Add study**.



3. Enter a name in the Study ID field (keep the Dispense volume at 150 µL as shown).
 - a. For FFPE tissues, select ***Bake and Dewax** as the Preparation protocol.
 - b. For fixed-frozen or fresh-frozen tissues, select ***----** instead.
4. Select **OK**.
5. Select **Add slide** to assign a protocol to each slide.



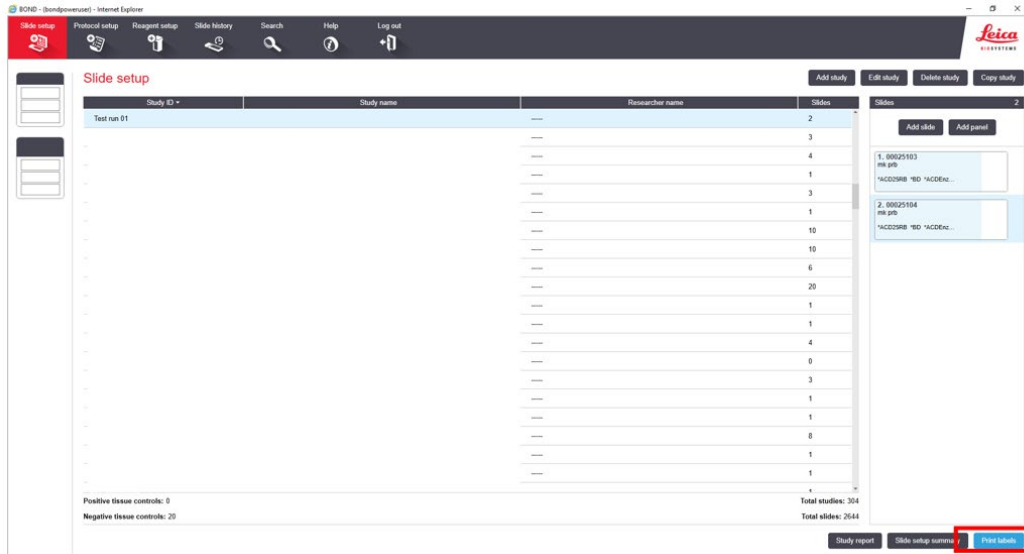
6. Enter the tissue type and probe name under the Comments field.
7. Keep **Single** as default from the Staining mode drop down menu.
8. Select **ISH** under Process and **Mock Probe (ACD)** from the Marker drop down menu.



+

9. Under the **Protocols** tab, do the following:
 - For *each* distinct probe, select an *applicable* protocol from the Staining drop down menu (for example, RNAscope Multiomic Protocol P1).
 - For standard FFPE tissues, select the protocol ***Bake and Dewax** from the Preparation drop down menu. For fixed or fresh frozen tissues, select ***----** instead.

- Select ***ACD HIER 15 min with ER2 (95)** as the HIER protocol or the appropriate HIER protocol for your tissue.
 - Select ***ACD 15 min Protease** for Enzyme, or the appropriate enzyme protocol for your tissue.
 - a. Select ***----** for Probe Application
 - Select ***----** for Denaturation
 - Select ***----** for Hybridization.
 - Select ***__** for Probe Removal
10. Select **Add slide** for each target probe and for each of the slides used in the run.
 11. After adding all the slides to the study, select **Close** to return to the Slide setup screen.
 12. Select **Print labels** to print barcodes to attach to the slides.

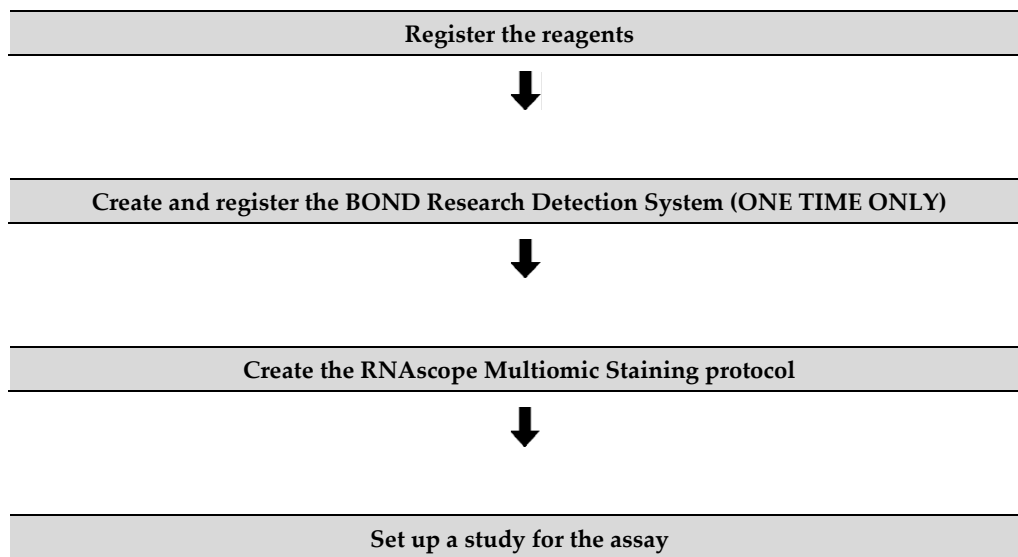


6

Chapter 6. Set Up a Staining Protocol without the Mock Probe

If you are staining RNA targets only, use the instructions in this chapter to set up the RNAscope Multiomic LS Assay without mock probe workaround recommended for LS software versions 6.0 and 7.0.

Workflow



Register the reagents

Your ACD Field Application Specialist (FAS) can help implement this procedure.

1. Select the **Reagent Setup** icon at the top of the screen.
2. Select **Add** to enter reagent information for components that are not pre-filled in the Leica software.
3. Enter a reagent name in the Name text box (see below Table)
4. Select **Ancillary** in the Type drop-down menu.
5. Enter **ACD** in the Supplier text box.
6. Check both the **Preferred** and **Hazardous** boxes (for RNAscope Multiomic Amp 1, and RNAscope Multiomic Amp 3 only).
7. Select **Save**.
8. Repeat Steps 2–7 for the rest of the reagents using the container names in the following table:

| Reagents | Container Name | Abbreviated Name | Volume per slide (dead volume not included) |
|-----------------------------|--------------------------|------------------|---|
| RNAscope Multiomic LS AMP 1 | RNAscope Multiomic Amp 1 | MOAmp1 | 300 µL |
| RNAscope Multiomic LS AMP 2 | RNAscope Multiomic Amp 2 | MOAmp2 | 300 µL |

| Reagents | Container Name | Abbreviated Name | Volume per slide (dead volume not included) |
|---|--|------------------|---|
| RNAscope Multiomic LS AMP 3 | RNAscope Multiomic Amp 3 | MOAmp3 | 300 µL |
| RNAscope Multiomic LS HRP C1 | RNAscope Multiomic HRP-C1 | MOHRPC1 | 300 µL |
| RNAscope Multiomic LS HRP C2 | RNAscope Multiomic HRP-C2 | MOHRPC2 | 300 µL |
| RNAscope Multiomic LS HRP C3 | RNAscope Multiomic HRP-C3 | MOHRPC3 | 300 µL |
| RNAscope Multiomic LS HRP C4 | RNAscope Multiomic HRP-C4 | MOHRPC4 | 300 µL |
| RNAscope Multiomic LS HRP C5 | RNAscope Multiomic HRP-C5 | MOHRPC5 | 300 µL |
| RNAscope Multiomic LS HRP C6 | RNAscope Multiomic HRP-C6 | MOHRPC6 | 300 µL |
| RNAscope Multiomic LS HRP Blocker | RNAscope Multiomic HRP Blocker | MOHRPbk | 1800 µL |
| Opal-fluorophore 1 (user to dilute in TSA buffer) | Multiomic TSA-F1 | MOTSAF1 | 300 µL |
| Opal-fluorophore 2 (user to dilute in TSA buffer) | Multiomic TSA-F2 | MOTSAF2 | 300 µL |
| Opal-fluorophore 3 (user to dilute in TSA buffer) | Multiomic TSA-F3 | MOTSAF3 | 300 µL |
| Opal-fluorophore 4 (user to dilute in TSA buffer) | Multiomic TSA-F4 | MOTSAF4 | 300 µL |
| Opal-fluorophore 5 (user to dilute in TSA buffer) | Multiomic TSA-F5 | MOTSAF5 | 300 µL |
| Opal TSA-DIG † (if Opal 780 is used) | TSA-DIG | TSA-DIG | 300 µL |
| Opal 780† (if Opal 780 is used) | Opal 780 | Opal780 | 300 µL |
| LS RNAscope Multiomic probe mix | (use any container but make sure that the container is registered as Probe RNA in the software) | — | 370 µL for 6.0/7.0 software |
| RNAscope Multiomic LS DAPI | DAPI | DAPI | 150 µL |
| BOND Wash | Bond Wash | BondWash | 150 µL |
| RNAscope Multiomic LS Hydrogen Peroxide | *RNAscope 2.5 LSx H2O2 | — | 150 µL |
| RNAscope Multiomic LS Protease III | *RNAscope 2.5 LSx Protease | — | 200 µL |
| RNAscope Multiomic LS Rinse | *LS Rinse | — | 600 µL |

*Indicates this reagent is hard coded in the software by Leica Biosystems.

†These reagents are only needed when Opal Polaris 780 is used in the assay.

Create and register the BOND Research Detection System (one time only)

A BOND Research Detection System from Leica is required to set up the RNAscope Multiomic LS Assay. Your ACD Field Application Specialist (FAS) should implement this procedure. Each detection system barcode is valid for up to 40 mL of use (equivalent to ~260 slides or four RNAscope Multiomic LS Fluorescent Reagent Kits).

1. Scan the barcode on the tray of a new BOND Research Detection System.
2. To setup a new detection system for the assay, enter **ACD LS Multiplex Detection Kit** in the Name text box.

Note: Creating the detection system needs to be performed only once on each BOND RX controller.

Add research reagent system

Name:

UPI:

Lot N°:

Expiration date:

Reagents

| Pstrn. | UPI | Reagent | Vol. (mL) |
|--------|-----|-----------|-----------|
| 1 | | DAPI | |
| 2 | | Bond Wash | |

[Add reagent](#) | [Remove reagent](#)

3. Place two new BOND 30 mL Open containers on the Research Detection System rack.
4. Scan the first container and select the registration name **DAPI**. You can mix different lots of DAPI in the same container.

Note: If you prefer not to use DAPI on the instrument or want to perform immunohistochemistry (IHC) steps after the assay, you may use BOND Wash in place of DAPI in the protocol.

5. Scan the second container and select the registration name **Bond Wash**.
6. When one Research Detection System is finished (up to 40 mL), register a new detection system by scanning the barcode on the tray and select **ACD LS Multiplex Detection Kit** from the drop-down menu on the right.
7. Select **Add**.

Create a staining protocol

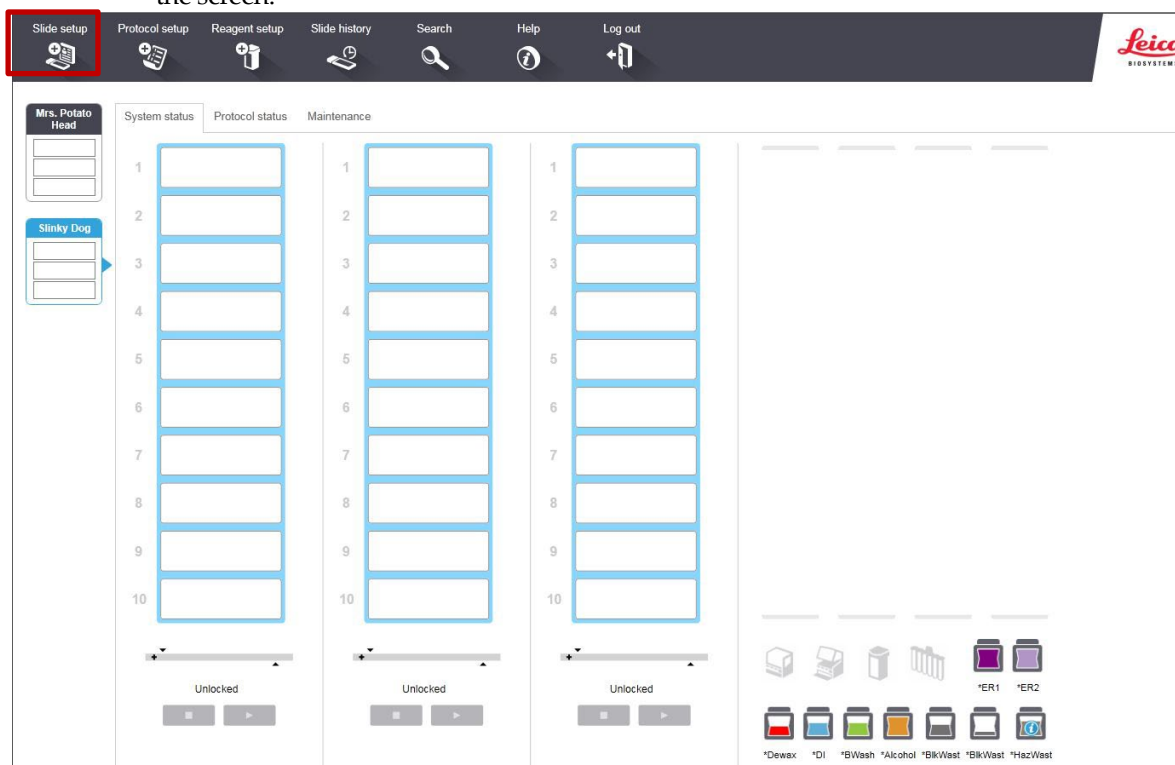
Your ACD Field Application Specialist (FAS) can help implement this procedure.

1. In the Protocol setup screen, select **Staining** under the Protocol drop down menu.

2. Highlight the ***ACD 2.5 DAB Rev B protocol**. Select **Copy**.
3. Change the protocol name to **ACD RNAscope Multiomic Protocol (or 6 mRNA)** in the Name text box, **Multi_P1** in the Abbreviated name text box, and **ACD RNAscope Multiomic Protocol** in the Description text box.
4. Select **ACD LS Multiplex Detection Kit** from the Preferred detection system menu.
5. Edit the protocol on-screen to match the protocol listed in Compare and confirm the on-screen protocol with the protocol listed in **Appendix B**.
6. Highlight and select each step to edit. Click **Show wash** steps to also view the wash steps. Insert **BOND Washes** to match each of the protocol steps shown.
7. Once the staining protocol matches the steps in **Appendix B**, delete steps 1–14. **ACD Multiomic Amp1** should be steps 1 and 2.
8. Select **Preferred** in the bottom right corner of the window. Select **Save**.

Set up a study for the RNAscope 6-plex LS Assay

1. To build a study for the RNAscope 6-plex LS Assay, select the **Slide setup** icon at the top of the screen.

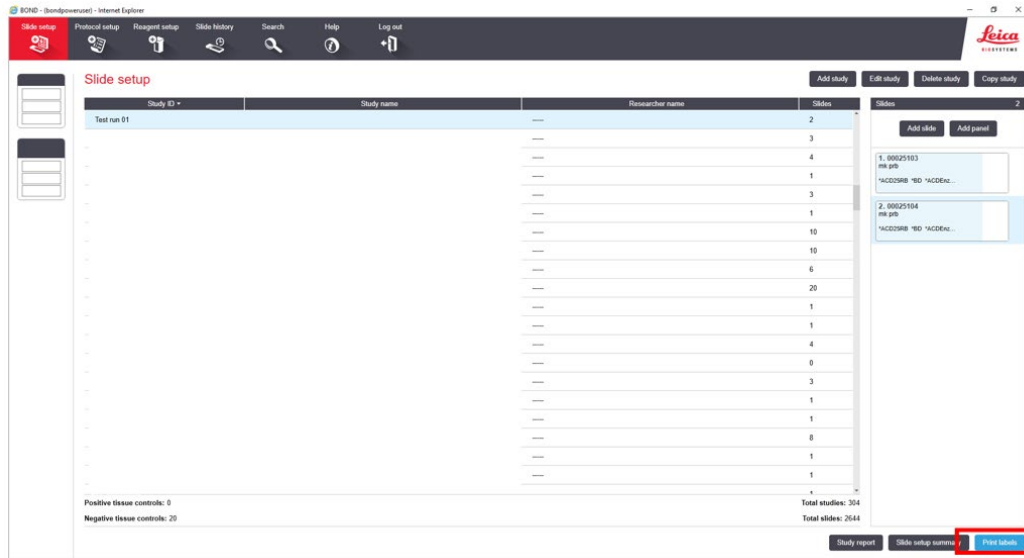


2. Select **Add study** and enter a name in the Study ID field (keep the Dispense volume at 150 μ L as shown).

3. For FFPE tissues, select ***Bake and Dewax** as the Preparation protocol. For frozen tissues, select ***----** instead.
4. Select **OK**.
5. Select **Add slide** to assign a protocol to each slide.
6. Enter the tissue type and probe name under the Comments field.
7. Keep **Single** as default from the Staining mode drop down menu.
8. Select **ISH** under Process and **the appropriate probe** from the Marker drop down menu.

9. Under the **Protocols** tab, do the following:

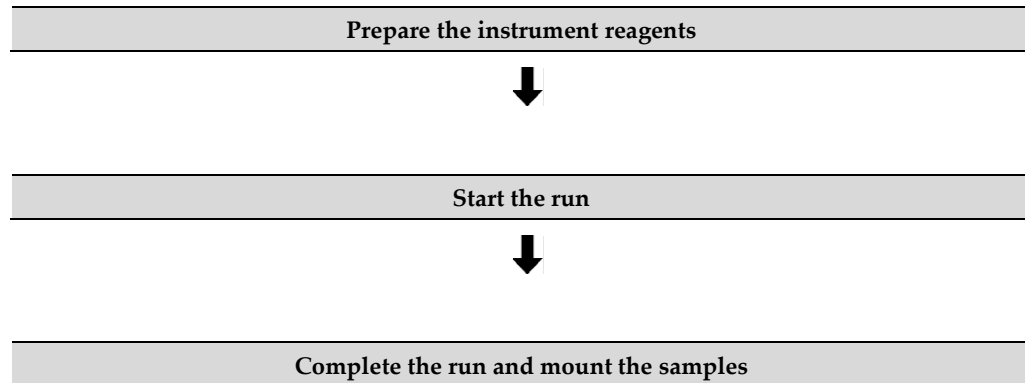
- a. Select the appropriate protocol from the Staining drop down menu (for example, ACD RNAscope Multiomic Protocol).
 - b. For standard FFPE tissues, select the protocol ***Bake and Dewax** from the Preparation drop down menu. For frozen tissues, select ***----** instead.
 - c. Select ***RNAscope 2.5 LSx Target Retrieval (88)** as the HIER protocol or the appropriate HIER protocol for your tissue.
 - d. Select ***RNAscope 2.5 LSx Enzyme**, or the appropriate enzyme protocol for your tissue. (if Pretreat Pro is being used it must be placed in a container and registered as LSx enzyme and used with an edited protocol; see Appendix K.
 - e. Select ***RNAscope 2.5 LSx Probe Application** from the Probe Application dropdown menu.
 - f. Select ***----** for Denaturation.
 - g. Select ***RNAscope 2.5 LSx Hybridization** for Hybridization.
 - h. Select ***RNAscope 2.5 LSx Probe Removal** for Probe Removal.
10. Select **Add slide** for each target probe and for each of the slides used in the run.
 11. After adding all the slides to the study, select **Close** to return to the Slide setup screen.
 12. Select **Print labels** to print barcodes to attach to the slides.



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Chapter 7. Run the Multiomic LS Assay

Workflow



Prepare the instrument reagents

1. Fill the DAPI container with DAPI and the Bond Wash container with Leica Biosystems' 1X BOND Wash. The kit requires 150 μL DAPI or Bond Wash per slide.
2. Carefully transfer all other RNAscope LS kit reagents *except for the TSA buffer* into empty 30 mL BOND Open containers.

Note: Before each run, make sure you have enough of each reagent. See the table on page 40 for the reagent volume required per slide.

IMPORTANT! Do not introduce bubbles into the solutions by shaking the containers. To mix reagents, gently invert the containers several times. If bubbles are present, leave the containers out at room temperature until the bubbles dissipate.

Note: You may use your own DAPI, or other counterstain, in place of the DAPI provided in the kit.

3. Prepare the RNAscope LS target probe mix:
 - a. Determine the volume of probe needed (volume needed for the total number of slides plus container dead-volume). For software 6.0/7.0, you will require 370 μL of probe per slide plus dead volume for the container. Make sure to add enough dead-volume to your calculation depending on the container type used:
 - 2.5 mL dead-volume when using a BOND 30 mL Open container.
 - 1 mL dead-volume when using a BOND 7 mL Open container.
 - 600 μL dead-volume when using a BOND Titration container (6 mL).
 - b. Dilute the 50X C2, C3, C4, C5 and C6 probe stocks 1:50 into the Ready-To-Use C1 probe. For example, add 320 μL of each of 50X C2, 50X C3, 50X C4, 50X C5 and 50X C6 probe to a tube, then add enough C1 probe to bring the final volume to 16 mL.
 - c. Transfer the RNAscope Multiomic LS probe mix into the appropriate Bond container, probe containers must be registered as **Probe RNA** in the software.

Note: The RNAscope probe mix is stable for one year at 2–8°C.

4. If performing an RNA + Protein staining, prepare conjugated antibodies:
 - a. Determine the volume of antibody mix needed (see Appendices C, D, and E for suggested concentration of each antibody) and add extra amount for dead volume of the container.
 - b. Dilute primary antibody conjugates together into one tube.
 - c. Dilute the antibody conjugates using the multiomic antibody diluent provided with the antibody.
 - d. If using a secondary conjugate, use a separate container to dilute it.

IMPORTANT! Do not pool secondaries with primary antibody conjugates.

1. Add diluted antibody conjugates to the appropriate Leica containers.
2. Prepare the Opal fluorophore dilutions:
 - a. Determine the volume of Opal fluorophore needed and make sure to add dead volume per container. Each slide requires 300 µL of fluorophore for staining.
 - b. Dilute the Opal fluorophore stock using the TSA buffer provided in the reagent kit.
 - c. Add the diluted fluorophores to the appropriate Leica containers.

| Reagents | Recommended dilution range | Dye Intensity |
|---------------------------------------|---|---------------|
| Opal 480* | 1:750–1:3000 (in TSA buffer) | Highest |
| Opal 520* | 1:750–1:3000 (in TSA buffer) | Highest |
| Opal 570* | 1:750–1:3000 (in TSA buffer) | Medium |
| Opal 620* | 1:750 -1:3000 (in TSA buffer) | Medium |
| Opal 690* | 1:750–1:3000 (in TSA buffer) | Low |
| Opal TSA-DIG* (if using Opal 780) | 1:750–1:3000 (in TSA buffer) | Lowest |
| Opal Polaris 780* (if using Opal 780) | 1:187.5–1:750+ (in Akoya or Bond diluent) | Lowest |

* Reconstitute all Opals (except Opal Polaris 780) with 75 µL Dimethylsulfoxide (DMSO). Reconstitute Opal Polaris 780 with 300 µL double distilled water (ddH₂O).

† We recommend keeping the dilution factors of Opal TSA-DIG and Opal Polaris 780 at a constant ratio. For example, when using 1:1500 dilution for Opal TSA-DIG, use 1:375 dilution for Opal Polaris 780. When using 1:750 dilution for Opal TSA-DIG, use 1:187.5 dilution for Opal Polaris 780.

5. For best results, assign brighter fluorophores to low expressors, develop high expressors last and low expressors first, and assign co-expressing markers to spectrally distinct fluorophores.

Scan containers into the software

1. Using the Barcode Scanner, scan the barcode located on the front of the BOND Open container. A window appears.
2. From the drop-down menu, select the corresponding name of the reagent as shown in the table on page 40 under **Container name**. Then click **Save**.

Prepare the instrument

1. Fill the large containers located in the bottom of the instrument with the Leica BOND RX bulk reagents.
2. Dilute BOND Wash Solution 1:10.

Note: Insufficient bulk reagent volumes may lead to run failure.

IMPORTANT! Do not introduce bubbles into the solutions by shaking the containers. To mix reagents, gently invert the containers several times. If bubbles are present, leave the containers out at room temperature until the bubbles dissipate.

3. Use clean, dry covertiles for every run. Follow Leica instructions to clean used covertiles with water, bleach, and ethanol. Air dry before reuse.
4. Before starting a run, empty bulk waste containers. Discard waste according to all local, state/provincial and/or national regulations.

Start the run

1. Attach the barcodes to the slides and add the slides to the slide tray with the label sides facing up.
2. Add a covertile on top of each slide and verify placement and seating of each covertile.

Note: The rectangular-shaped neck of the covertile should fit into the groove of the slide tray.

3. Place the tray in the Leica BOND RX and press the button to load the tray onto the machine.
4. Once the slides have been scanned, select the **PLAY** (triangular) button on the screen located under the start tray to start the run. Alternatively, right-click on scanned label images, and select **Delayed Start** to start the run at a future time. Do not use Delayed Start with fixed or freshfrozen tissue.

Note: The total run time for LS RNAscope Multiomic Fluorescent Assay is 14–15 hrs depending on the number of slides.

IMPORTANT! Before leaving the instrument unattended, ensure that the instrument is running successfully.

Complete the run and mount the samples

1. After the run is complete, press the button on the front of the instrument to unload the slides.
2. Remove the covertiles.
3. Add a drop of ProLong Gold Antifade Mountant to each slide. Avoid introducing bubbles.
4. Carefully place a glass coverslip on the slides, and dry overnight in the dark.
5. Store the slides at 4°C in the dark for up to two weeks.

8

Chapter 8. Evaluate the Results

Evaluate the samples

Examine tissue sections under a standard fluorescent microscope at 20–40X magnification. You may also use a confocal microscope.

- Assess tissue and cell morphology.
- Assess the negative control background first. One dot to every 10 cells displaying background staining per 20X microscope field is acceptable. Set the light source and exposure time of image acquisition to acceptable background levels.
- Assess positive control signal strength. Positive control signal should be visible as punctate dots within the cell at 20–40X magnification.

Scoring Guidelines

When used for RNA detection, the assay enables a semiquantitative scoring guideline utilizing the estimated number of punctate dots present within each cell boundary. An example of how to develop such a guideline for semi-quantitative assessment of mRNA staining intensity is presented below for a gene with expression level varying between 1 to > 10 dots per cell.

Note: If your gene expression level is higher or lower than this range, you may need to scale the criteria accordingly.

mRNA scoring is divided into five categories:

| Staining Score | Microscope Objective Scoring |
|----------------|--|
| 0 | No staining or less than 1 dot per 10 cells |
| 1 | 1–3 dots per cell |
| 2 | 4–9 dots per cell, no or very few dot clusters |
| 3 | 10–15 dots per cell and/or <10% dots are in clusters |
| 4 | >15 dots per cell and/or >10% dots are in clusters |

Control examples

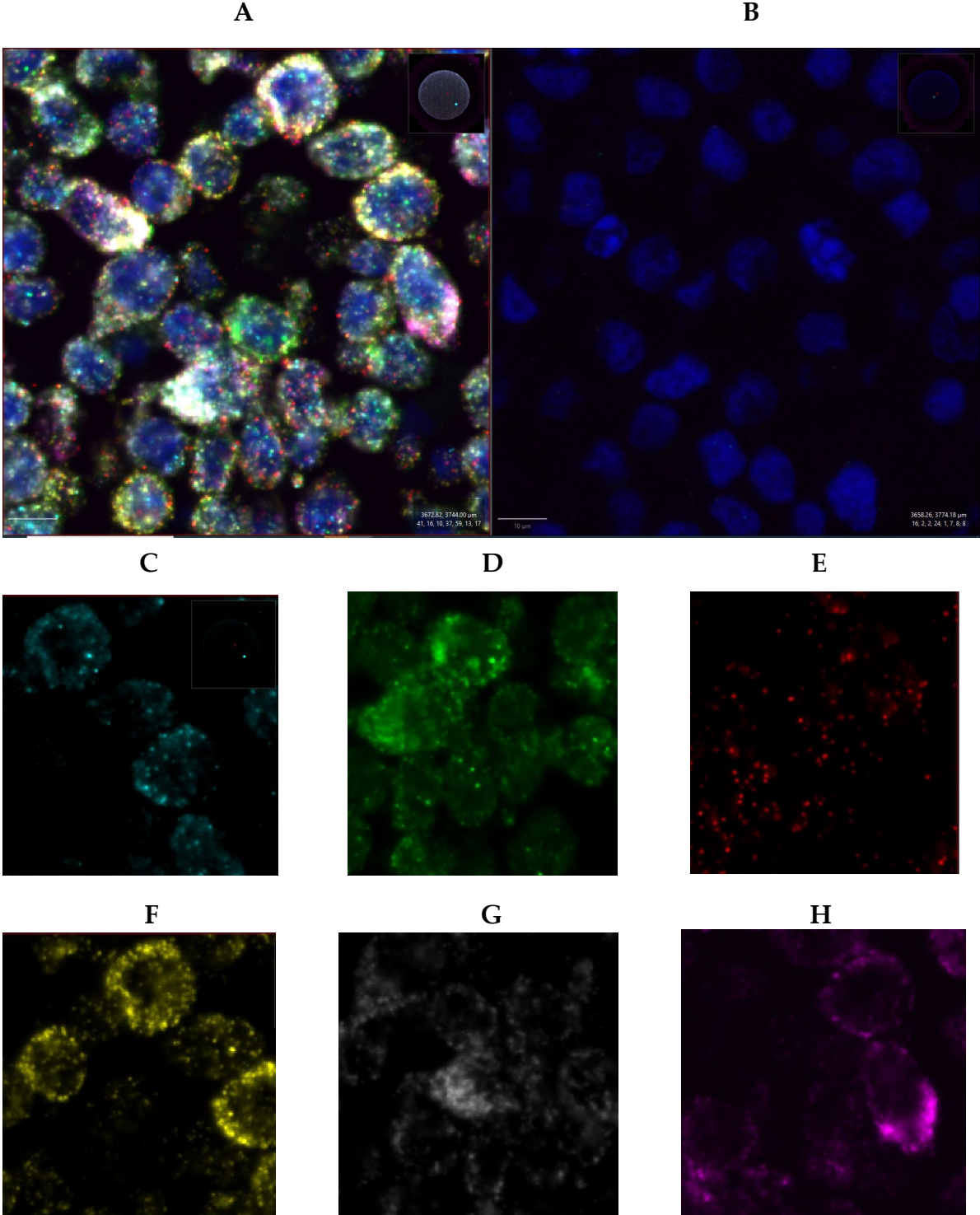


Figure 2: mRNA staining examples from FFPE HeLa cell pellet using RNAscope Multiomic LS Fluorescent Assay at 40X magnification. **A)** Positive control slide showing 6 RNA targets: *POLR2A* (teal), *ACTB* (green), *HPRT1* (red), *UBC* (yellow), *PPIB* (white), *TUBB* (pink). **B)** Negative control slide with *dapB*. **C-H)** Individual channels at higher magnification.

If the assay is successful, the staining should look like the following image:

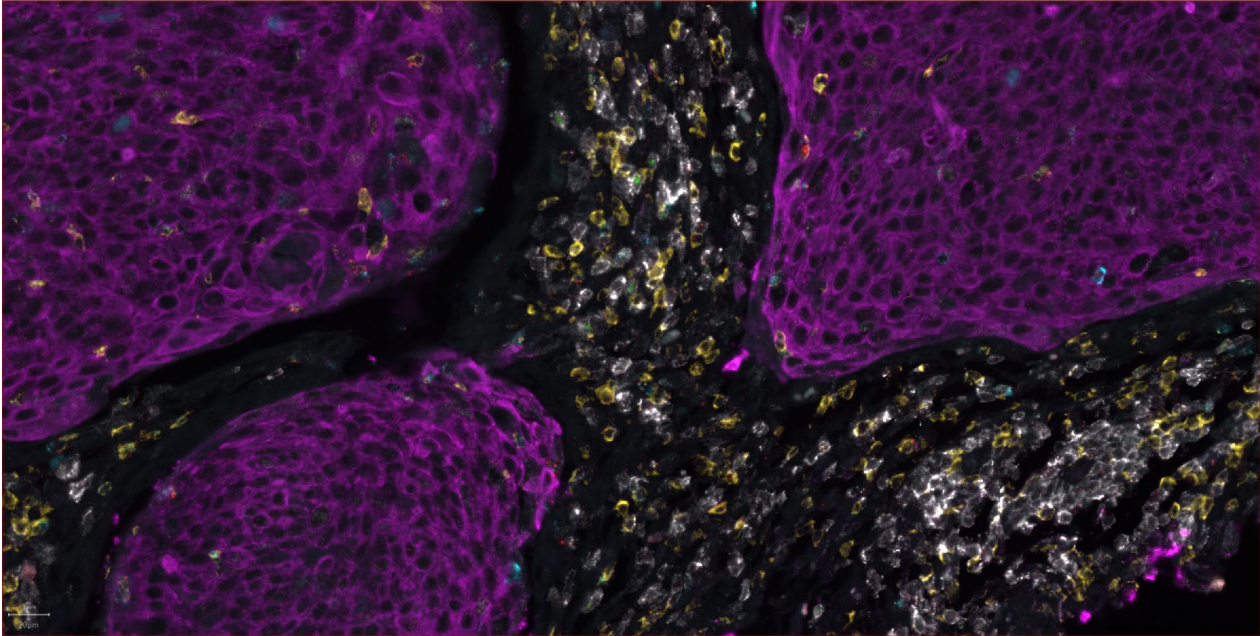


Figure 3. RNAscope Multiomic LS Fluorescent Assay detection of 2 RNAs and 3 proteins. *IFN* γ (red) *GZMB* (blue) CD4 (yellow) CD8a (white) PanCK (pink) in Cervical cancer FFPE tissue at 20X magnification.

Troubleshooting

If you obtain less than satisfactory results, troubleshoot your assay by following these simple guidelines:

- Always use optimal fluorescent filter settings and imaging tools.
 - If signal intensity is too low for your imaging tools, increase the fluorophore concentration.
 - Use optimized fluorescence filter sets to reduce signal bleed-through. If you observe fluorescence bleed-through, reduce the fluorophore concentration of the bleeding-through channel and/or reduce the exposure time during image acquisition to avoid over-exposure.
 - If your RNA ISH signal cannot be distinguished from autofluorescence in tissues with high autofluorescence, increase the fluorophore concentration.
 - If you observe the presence of background staining, limit the sensitivity of image acquisition or reduce the corresponding Opal fluorophore concentration. Always acquire images using the setting in which background is under-detected.
 - If the signal-to-noise ratio is low due to high background, optimize pretreatment conditions. Contact ACD support for recommendations.
 - The RNAscope RNAscope Multiomic LS Fluorescence Assay uses only the Leica Biosystem BOND Research Detection System. Do not use BOND Polymer Refine DAB/Red Detection kits or any other chromogen kits.
 - Do not shake the contents in the dispensers as this will form bubbles and may lead to weak or no staining. If bubbles are present, leave the containers out at room temperature until the bubbles dissipate.
 - **Target retrieval for 20min at 100°C can be used if dye trapping or lower antibody signal is observed.**
 - For troubleshooting information, please contact technical support at support.acd@bio-techne.com.



Appendix A. Protease-Free RNA 6-plex Staining Protocol (using the Mock Probe workaround)

You can perform a 6-plex RNA-only run using the workflow described in Chapter 6, which does not use the Mock Probe Reagent.

IMPORTANT! Heated *Bond Wash solution steps come from the bulk reagents and are heated by the instrument. You cannot delete these steps. You may delete other wash steps.

IMPORTANT! The BOND RX can only execute this workflow on one tray for the RNAscope Multiomic assay (six channels) due to its length, follow the protocol steps listed in the following table. Adding any additional steps will prevent the system from running even one trays.

Slide Setup:

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-------------------------|-----------|-----------------|--------------|
| 1 | RNAscope LS PretreatPro | Reagent | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|---|----------------|-----------------|--------------|
| 2 | RNAscope LS PretreatPro | Reagent | 30 MIN | 40°C |
| 3 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 4 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 5 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 6 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 7 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 8 | RNAscope Multiomic LS Hydrogen Peroxide | Reagent | 10 MIN | Ambient |
| 9 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 10 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 11 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 12 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 13 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 14 | Probe 1 | Reagent | 0 MIN | Ambient |
| 15 | Probe 1 | Reagent | 120 MIN | 42°C |
| 16 | *Bond Wash Solution | Wash | 0 MIN | 42°C |
| 17 | *Bond Wash Solution | Wash | 1 MIN | 42°C |
| 18 | *Bond Wash Solution | Wash | 5 MIN | 42°C |
| 19 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 20 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 21 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 22 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 23 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 24 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 25 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 26 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 27 | RNAscope Multiomic LS Amp 1 | Reagent | 1 MIN | 42°C |
| 28 | RNAscope Multiomic LS Amp 1 | Reagent | 30 MIN | 42°C |
| 29 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 30 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 31 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 32 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 33 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 34 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 35 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 36 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 37 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 38 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 39 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|------------------------------|-----------|-----------------|--------------|
| 40 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 41 | *Bond Wash Solution | Open Wash | 0 MIN | Ambient |
| 42 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 43 | RNAscope Multiomic LS Amp 2 | Reagent | 1 MIN | 42°C |
| 44 | RNAscope Multiomic LS Amp 2 | Reagent | 30 MIN | 42°C |
| 45 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 46 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 47 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 48 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 49 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 50 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 51 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 52 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 53 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 54 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 55 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 56 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 57 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 58 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 59 | RNAscope Multiomic LS Amp 3 | Reagent | 1 MIN | 42°C |
| 60 | RNAscope Multiomic LS Amp 3 | Reagent | 15 MIN | 42°C |
| 61 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 62 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 63 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 64 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 65 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 66 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 67 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 68 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 69 | RNAscope Multiomic LS HRP C1 | Reagent | 1 MIN | 42°C |
| 70 | RNAscope Multiomic LS HRP C1 | Reagent | 15 MIN | 42°C |
| 71 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 72 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 73 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 74 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 75 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 76 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 77 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 78 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------------|-----------|-----------------|--------------|
| 79 | RNAscope Multiomic TSA-F1 | Reagent | 1 MIN | Ambient |
| 80 | RNAscope Multiomic TSA-F1 | Reagent | 30 MIN | Ambient |
| 81 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 82 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 83 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 84 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 85 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 86 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 87 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 88 | RNAscope Multiomic LS HRP Blocker | Reagent | 1 MIN | 42°C |
| 89 | RNAscope Multiomic LS HRP Blocker | Reagent | 15 MIN | 42°C |
| 90 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 91 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 92 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 93 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 94 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 95 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 96 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 97 | RNAscope Multiomic HRP C2 | Reagent | 1 MIN | 42°C |
| 98 | RNAscope Multiomic HRP C2 | Reagent | 15 MIN | 42°C |
| 99 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 100 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 101 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 102 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 103 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 104 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 105 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 106 | RNAscope Multiomic TSA-F2 | Reagent | 1 MIN | Ambient |
| 107 | RNAscope Multiomic TSA-F2 | Reagent | 30 MIN | Ambient |
| 108 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 109 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 110 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 111 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 112 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 113 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 114 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 115 | RNAscope Multiomic LS HRP Blocker | Reagent | 1 MIN | 42°C |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------------|-----------|-----------------|--------------|
| 116 | RNAscope Multiomic LS HRP Blocker | Reagent | 15 MIN | 42°C |
| 117 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 118 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 119 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 120 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 121 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 122 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 123 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 124 | RNAscope Multiomic LS HRP C3 | Reagent | 1 MIN | 42°C |
| 125 | RNAscope Multiomic LS HRP C3 | Reagent | 15 MIN | 42°C |
| 126 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 127 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 128 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 129 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 130 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 131 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 132 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 133 | RNAscope Multiomic TSA-F3 | Reagent | 1 MIN | Ambient |
| 134 | RNAscope Multiomic TSA-F3 | Reagent | 30 MIN | Ambient |
| 135 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 136 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 137 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 138 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 139 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 140 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 141 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 142 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 143 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 144 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 145 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 146 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 147 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 148 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 149 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 150 | RNAscope Multiomic LS HRP C4 | Reagent | 1 MIN | 42°C |
| 151 | RNAscope Multiomic LS HRP C4 | Reagent | 15 MIN | 42°C |
| 152 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 153 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------------|-----------|-----------------|--------------|
| 154 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 155 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 156 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 157 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 158 | RNAscope Multiomic TSA-F4 | Reagent | 1 MIN | Ambient |
| 159 | RNAscope Multiomic TSA-F4 | Reagent | 30 MIN | Ambient |
| 160 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 161 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 162 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 163 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 164 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 165 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 166 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 167 | RNAscope Multiomic LS HRP Blocker | Reagent | 1 MIN | 42°C |
| 168 | RNAscope Multiomic LS HRP Blocker | Reagent | 15 MIN | 42°C |
| 169 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 170 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 171 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 172 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 173 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 174 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 175 | RNAscope Multiomic LS HRP C5 | Reagent | 1 MIN | 42°C |
| 176 | RNAscope Multiomic LS HRP C5 | Reagent | 15 MIN | 42°C |
| 177 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 178 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 179 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 180 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 181 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 182 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 183 | RNAscope Multiomic TSA-F5 | Reagent | 1 MIN | Ambient |
| 184 | RNAscope Multiomic TSA-F5 | Reagent | 30 MIN | Ambient |
| 185 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 186 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 187 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 188 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 189 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 190 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------------|-----------|-----------------|--------------|
| 191 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 192 | RNAscope Multiomic LS HRP Blocker | Reagent | 1 MIN | 42°C |
| 193 | RNAscope Multiomic LS HRP Blocker | Reagent | 15 MIN | 42°C |
| 194 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 195 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 196 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 197 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 198 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 199 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 200 | RNAscope Multiomic LS HRP C6 | Reagent | 1 MIN | 42°C |
| 201 | RNAscope Multiomic LS HRP C6 | Reagent | 15 MIN | 42°C |
| 202 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 203 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 204 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 205 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 206 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 207 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 208 | Opal TSA-DIG† | Reagent | 1 MIN | Ambient |
| 209 | Opal TSA-DIG† | Reagent | 30 MIN | Ambient |
| 210 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 211 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 212 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 213 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 214 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 215 | RNAscope Multiomic LS HRP Blocker | Reagent | 1 MIN | 42°C |
| 216 | RNAscope Multiomic LS HRP Blocker | Reagent | 15 MIN | 42°C |
| 217 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 218 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 219 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 220 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 221 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 222 | Polaris 780 | Reagent | 1 MIN | Ambient |
| 223 | Polaris 780 | Reagent | 30 MIN | Ambient |
| 224 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 225 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 226 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|----------------------------|-----------|-----------------|--------------|
| 227 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 228 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 229 | RNAscope Multiomic LS DAPI | Reagent | 10 MIN | Ambient |
| 230 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 231 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 232 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

* Indicates reagent is hard coded in the software by Leica Biosystems.

† If Opal 780 is used

‡ The standard protocol uses DAPI. Use BOND Wash instead of DAPI, if you are using DAPI offline or performing IHC steps afterwards on your samples.

B

Appendix B. mRNA only 6-plex Protocol (using the Mock Probe workaround)

Heated bond washes 3-5 come from the bulk reagents and are heated by the instrument. You cannot delete these steps. You may delete other wash steps.

IMPORTANT! The BONDRX can only execute this workflow on one tray for the RNAscope Multiomic assay (six channels) due to its length, follow the protocol steps listed in the following table. Adding any additional steps will prevent the system from running even one the tray.

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 1 | Probe 1 | Reagent | 0 MIN | Ambient |
| 2 | Probe 1 | Reagent | 120 MIN | 42°C |
| 3 | *Bond Wash Solution | Wash | 0 MIN | 42°C |
| 4 | *Bond Wash Solution | Wash | 1 MIN | 42°C |
| 5 | *Bond Wash Solution | Wash | 5 MIN | 42°C |
| 6 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 7 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 8 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 9 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 10 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 11 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 12 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 13 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 14 | RNAscope Multiomic LS Amp 1 | Reagent | 1 MIN | 42°C |
| 15 | RNAscope Multiomic LS Amp 1 | Reagent | 30 MIN | 42°C |
| 16 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 17 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 18 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 19 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 20 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 21 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 22 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 23 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 24 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 25 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 26 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 27 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 28 | *Bond Wash Solution | Open Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|------------------------------|-----------|-----------------|--------------|
| 29 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 30 | RNAscope Multiomic LS Amp 2 | Reagent | 1 MIN | 42°C |
| 31 | RNAscope Multiomic LS Amp 2 | Reagent | 30 MIN | 42°C |
| 32 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 33 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 34 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 35 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 36 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 37 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 38 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 39 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 40 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 41 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 42 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 43 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 44 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 45 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 46 | RNAscope Multiomic LS Amp 3 | Reagent | 1 MIN | 42°C |
| 47 | RNAscope Multiomic LS Amp 3 | Reagent | 15 MIN | 42°C |
| 48 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 49 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 50 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 51 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 52 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 53 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 54 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 55 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 56 | RNAscope Multiomic LS HRP C1 | Reagent | 1 MIN | 42°C |
| 57 | RNAscope Multiomic LS HRP C1 | Reagent | 15 MIN | 42°C |
| 58 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 59 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 60 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 61 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 62 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 63 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 64 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 65 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 66 | RNAscope Multiomic TSA-F1 | Reagent | 1 MIN | Ambient |
| 67 | RNAscope Multiomic TSA-F1 | Reagent | 30 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 68 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 69 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 70 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 71 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 72 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 73 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 74 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 75 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 76 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 77 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 78 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 79 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 80 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 81 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 82 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 83 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 84 | RNAscope Multiomic LS HRP C2 | Reagent | 1 MIN | 42°C |
| 85 | RNAscope Multiomic LS HRP C2 | Reagent | 15 MIN | 42°C |
| 86 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 87 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 88 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 89 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 90 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 91 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 92 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 93 | RNAscope Multiomic TSA-F2 | Reagent | 1 MIN | Ambient |
| 94 | RNAscope Multiomic TSA-F2 | Reagent | 30 MIN | Ambient |
| 95 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 96 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 97 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 98 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 99 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 100 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 101 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 102 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 103 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 104 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 105 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 106 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 107 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 108 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 109 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 110 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 111 | RNAscope Multiomic LS HRP C3 | Reagent | 1 MIN | 42°C |
| 112 | RNAscope Multiomic LS HRP C3 | Reagent | 15 MIN | 42°C |
| 113 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 114 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 115 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 116 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 117 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 118 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 119 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 120 | RNAscope Multiomic TSA-F3 | Reagent | 1 MIN | Ambient |
| 121 | RNAscope Multiomic TSA-F3 | Reagent | 30 MIN | Ambient |
| 122 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 123 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 124 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 125 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 126 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 127 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 128 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 129 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 130 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 131 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 132 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 133 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 134 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 135 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 136 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 137 | RNAscope Multiomic LS HRP C4 | Reagent | 1 MIN | 42°C |
| 138 | RNAscope Multiomic LS HRP C4 | Reagent | 15 MIN | 42°C |
| 139 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 140 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 141 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 142 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 143 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 144 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 145 | RNAscope Multiomic TSA-F4 | Reagent | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 146 | RNAscope Multiomic TSA-F4 | Reagent | 30 MIN | Ambient |
| 147 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 148 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 149 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 150 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 151 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 152 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 153 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 154 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 155 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 156 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 157 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 158 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 159 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 160 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 161 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 162 | RNAscope Multiomic LS HRP C5 | Reagent | 1 MIN | 42°C |
| 163 | RNAscope Multiomic LS HRP C5 | Reagent | 15 MIN | 42°C |
| 164 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 165 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 166 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 167 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 168 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 169 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 170 | RNAscope Multiomic TSA-F5 | Reagent | 1 MIN | Ambient |
| 171 | RNAscope Multiomic TSA-F5 | Reagent | 30 MIN | Ambient |
| 172 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 173 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 174 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 175 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 176 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 177 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 178 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 179 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 180 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 181 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 182 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 183 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 184 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 185 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 186 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 187 | RNAscope Multiomic LS HRP C6 | Reagent | 1 MIN | 42°C |
| 188 | RNAscope Multiomic LS HRP C6 | Reagent | 15 MIN | 42°C |
| 189 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 190 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 191 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 192 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 193 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 194 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 195 | TSA-DIG | Reagent | 1 MIN | Ambient |
| 196 | TSA-DIG | Reagent | 30 MIN | Ambient |
| 197 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 198 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 199 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 200 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 201 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 202 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 203 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 204 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 205 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 206 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 207 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 208 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 209 | Polaris 780 | Reagent | 1 MIN | Ambient |
| 210 | Polaris 780 | Reagent | 30 MIN | Ambient |
| 211 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 212 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 213 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 214 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 215 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 216 | RNAscope Multiomic LS DAPI | Reagent | 10 MIN | Ambient |
| 217 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 218 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 219 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

* Indicates reagent is hard coded in the software by Leica Biosystems.

‡ The standard protocol uses DAPI. Use BOND Wash instead of DAPI, if you are using DAPI offline or performing IHC steps afterwards on your samples.



Appendix C. mRNA and conjugated primary antibody detection

| Reagent | Container name | Details (concentration, dilution) |
|------------------|------------------------|---|
| Salmon Sperm DNA | Open1 | 500ug/mL, in multiomic antibody diluent |
| 10% NBF | 10% NBF | None |
| RNA pooled Probe | Open 2 | 1:50, in RNAscope probe diluent |
| Antibody mix | CoDetection Antibody 1 | Multiomic antibody diluent (See Appendix H for concentration) |

Slide setup:

Slide properties x

Pos 1

Tissue type: **Dispense volume:**

Test tissue 100 µL
 Negative tissue 150 µL
 Positive tissue

Staining mode:

Single Routine

Single

Process: IHC ISH

Marker: Mock Probe

Protocols

Staining: mRNA + Primary Conjugates

Preparation: *Bake and Dewax

HIER: *ACD HIER 15 min with ER2 (95)

Enzyme: *----

Probe Application: *RNAscope 2.5 LSx Probe Application

Denaturation: *----

Hybridization: ACD 1 Min Hybridization

Probe Removal: *RNAscope 2.5 LSx Probe Removal

OK
Copy slide
Close

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 1 | Open1 | Reagent | 0 MIN | Ambient |
| 2 | Open1 | Reagent | 60 MIN | Ambient |
| 3 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 4 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 5 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 6 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 7 | Co-Detection Antibody 1 | Reagent | 0 MIN | Ambient |
| 8 | Co-Detection Antibody 1 | Reagent | 60 MIN | Ambient |
| 9 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 10 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 11 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 12 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 13 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 14 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 15 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 16 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 17 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 18 | *Bond Wash Solution | Open Wash | 0 MIN | Ambient |
| 19 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 20 | 10% NBF | Reagent | 30 MIN | Ambient |
| 21 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 22 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 23 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 24 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 25 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 26 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 27 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 28 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 29 | PretreatPro | Reagent | 0 MIN | Ambient |
| 30 | PretreatPro | Reagent | 30 MIN | 40°C |
| 31 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 32 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 33 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 34 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 35 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 36 | Hydrogen Peroxide | Reagent | 10 MIN | Ambient |
| 37 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 38 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 39 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 40 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 41 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 42 | Open 2 | Reagent | 0 MIN | Ambient |
| 43 | Open 2 | Reagent | 120 MIN | 42°C |
| 44 | *Bond Wash Solution | Wash | 0 MIN | 42°C |
| 45 | *Bond Wash Solution | Wash | 1 MIN | 42°C |
| 46 | *Bond Wash Solution | Wash | 5 MIN | 42°C |
| 47 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 48 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 49 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 50 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 51 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 52 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 53 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 54 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 55 | RNAscope Multiomic Amp 1 | Reagent | 1 MIN | 42°C |
| 56 | RNAscope Multiomic Amp 1 | Reagent | 30 MIN | 42°C |
| 57 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 58 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 59 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 60 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 61 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 62 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 63 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 64 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 65 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 66 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 67 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 68 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 69 | *Bond Wash Solution | Open Wash | 0 MIN | Ambient |
| 70 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 71 | RNAscope Multiomic Amp 2 | Reagent | 1 MIN | 42°C |
| 72 | RNAscope Multiomic Amp 2 | Reagent | 30 MIN | 42°C |
| 73 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 74 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 75 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 76 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 77 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 78 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 79 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 80 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 81 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 82 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 83 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 84 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 85 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 86 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 87 | RNAscope Multiomic Amp 3 | Reagent | 1 MIN | 42°C |
| 88 | RNAscope Multiomic Amp 3 | Reagent | 15 MIN | 42°C |
| 89 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 90 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 91 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 92 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 93 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 94 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 95 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 96 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 97 | RNAscope Multiomic HRP-C1 | Reagent | 1 MIN | 42°C |
| 98 | RNAscope Multiomic HRP-C1 | Reagent | 15 MIN | 42°C |
| 99 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 100 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 101 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 102 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 103 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 104 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 105 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 106 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 107 | RNAscope Multiomic TSA-F1 | Reagent | 1 MIN | Ambient |
| 108 | RNAscope Multiomic TSA-F1 | Reagent | 30 MIN | Ambient |
| 109 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 110 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 111 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 112 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 113 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 114 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 115 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 116 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 117 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 118 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 119 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 120 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 121 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 122 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 123 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 124 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 125 | RNAscope Multiomic LS HRP C2 | Reagent | 1 MIN | 42°C |
| 126 | RNAscope Multiomic LS HRP C2 | Reagent | 15 MIN | 42°C |
| 127 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 128 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 129 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 130 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 131 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 132 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 133 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 134 | RNAscope Multiomic TSA-F2 | Reagent | 1 MIN | Ambient |
| 135 | RNAscope Multiomic TSA-F2 | Reagent | 30 MIN | Ambient |
| 136 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 137 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 138 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 139 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 140 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 141 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 142 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 143 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 144 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 145 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 146 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 147 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 148 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 149 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 150 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 151 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 152 | RNAscope Multiomic LS HRP C3 | Reagent | 1 MIN | 42°C |
| 153 | RNAscope Multiomic LS HRP C3 | Reagent | 15 MIN | 42°C |
| 154 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 155 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 156 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 157 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 158 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 159 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 160 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 161 | RNAscope Multiomic TSA-F3 | Reagent | 1 MIN | Ambient |
| 162 | RNAscope Multiomic TSA-F3 | Reagent | 30 MIN | Ambient |
| 163 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 164 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 165 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 166 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 167 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 168 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 169 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 170 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 171 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 172 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 173 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 174 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 175 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 176 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 177 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 178 | RNAscope Multiomic LS HRP C4 | Reagent | 1 MIN | 42°C |
| 179 | RNAscope Multiomic LS HRP C4 | Reagent | 15 MIN | 42°C |
| 180 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 181 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 182 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 183 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 184 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 185 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 186 | RNAscope Multiomic TSA-F4 | Reagent | 1 MIN | Ambient |
| 187 | RNAscope Multiomic TSA-F4 | Reagent | 30 MIN | Ambient |
| 188 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 189 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 190 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 191 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 192 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 193 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 194 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 195 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 196 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 197 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 198 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 199 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 200 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 201 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 202 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 203 | RNAscope Multiomic LS HRP C5 | Reagent | 1 MIN | 42°C |
| 204 | RNAscope Multiomic LS HRP C5 | Reagent | 15 MIN | 42°C |
| 205 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 206 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 207 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 208 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 209 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 210 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 211 | RNAscope Multiomic TSA-F5 | Reagent | 1 MIN | Ambient |
| 212 | RNAscope Multiomic TSA-F5 | Reagent | 30 MIN | Ambient |
| 213 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 214 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 215 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 216 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 217 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 218 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 219 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 220 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 221 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 222 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 223 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 224 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 225 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 226 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 227 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 228 | RNAscope Multiomic LS HRP C6 | Reagent | 1 MIN | 42°C |
| 229 | RNAscope Multiomic LS HRP C6 | Reagent | 15 MIN | 42°C |
| 230 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 231 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 232 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 233 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 234 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 235 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 236 | TSA-DIG | Reagent | 1 MIN | Ambient |
| 237 | TSA-DIG | Reagent | 30 MIN | Ambient |
| 238 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 239 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 240 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 241 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 242 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 243 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 244 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 245 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 246 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 247 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 248 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 249 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 250 | Polaris 780 | Reagent | 1 MIN | Ambient |
| 251 | Polaris 780 | Reagent | 30 MIN | Ambient |
| 252 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 253 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 254 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 255 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 256 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 257 | DAPI | Reagent | 10 MIN | Ambient |
| 258 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 259 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 260 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

D

Appendix D. mRNA + secondary antibody + Primary conjugated antibodies

| | Reagent | Container name | Details (concentration, dilution) |
|---|--|------------------------|---|
| 1 | Salmon Sperm DNA | Open1 | 500ug/ml, in multiomic antibody diluent |
| 2 | 10% NBF | 10% NBF | None |
| 3 | RNA Probe | Open 2 | 1:50, in RNAscope probe diluent |
| 4 | Primary Raw Antibody mix | CoDetection Antibody 1 | Multiomic antibody diluent (user defined) |
| 5 | Secondary Conjugated Antibody mix | CoDetection Antibody 2 | Multiomic antibody diluent (See Appendix H for concentration) |
| 6 | Primary Conjugated Antibody mix | CoDetection Antibody 3 | Multiomic antibody diluent (See Appendix H for concentration) |
| 7 | Antibody Blocker (mouse and/or Rabbit IgG) | Antibody Blocker | 5ug/ml, in multiomic antibody diluent |

IMPORTANT: When using the RNAscope conjugated secondary antibodies, they may bind to the RNAscope primary conjugated antibodies. To avoid cross-detection, addition of antibody blocker, 5ug/ml of each mouse and rabbit IgG (Mouse IgG2A Isotype Control (R&D Systems MAB003) and Normal Rabbit IgG Control (R&D Systems MAB1050) in multiomic antibody diluent for 30min at RT between the incubation with secondary conjugated antibody and incubation with conjugated primary antibody is required. This will help eliminate this cross-reactivity. The IgG blocker may be added in the “Antibody Blocker” in steps 19 and 20 below.

Slide setup:

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-------------------------|-----------|-----------------|--------------|
| 1 | Open1 | Reagent | 0 MIN | Ambient |
| 2 | Open1 | Reagent | 60 MIN | Ambient |
| 3 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 4 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 5 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 6 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 7 | Co-Detection Antibody 1 | Reagent | 0 MIN | Ambient |
| 8 | Co-Detection Antibody 1 | Reagent | 60 MIN | Ambient |
| 9 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 10 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 11 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 12 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 13 | Co-Detection Antibody 2 | Reagent | 0 MIN | Ambient |
| 14 | Co-Detection Antibody 2 | Reagent | 30 MIN | Ambient |
| 15 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 16 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 17 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 18 | *Bond Wash Solution | Wash | 1MIN | Ambient |
| 19 | Antibody Blocker | Reagent | 0 MIN | Ambient |
| 20 | Antibody Blocker | Reagent | 30 MIN | Ambient |
| 21 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 22 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 23 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 24 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 25 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 26 | Co-Detection Antibody 3 | Reagent | 0 MIN | Ambient |
| 27 | Co-Detection Antibody 3 | Reagent | 60 MIN | Ambient |
| 28 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 29 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 30 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 31 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 32 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 33 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 34 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 35 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 36 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 37 | *Bond Wash Solution | Open Wash | 0 MIN | Ambient |
| 38 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 39 | 10% NBF | Reagent | 30 MIN | Ambient |
| 40 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 41 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 42 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 43 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 44 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 45 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 46 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 47 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 48 | PretreatPro | Reagent | 0 MIN | Ambient |
| 49 | PretreatPro | Reagent | 30 MIN | 40°C |
| 50 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 51 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 52 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 53 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 54 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 55 | Hydrogen Peroxide | Reagent | 10 MIN | Ambient |
| 56 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 57 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 58 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 59 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 60 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 61 | Open 2 | Reagent | 0 MIN | Ambient |
| 62 | Open 2 | Reagent | 120 MIN | 42°C |
| 63 | *Bond Wash Solution | Wash | 0 MIN | 42°C |
| 64 | *Bond Wash Solution | Wash | 1 MIN | 42°C |
| 65 | *Bond Wash Solution | Wash | 5 MIN | 42°C |
| 66 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 67 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 68 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 69 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 70 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 71 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 72 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 73 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 74 | RNAscope Multiomic Amp 1 | Reagent | 1 MIN | 42°C |
| 75 | RNAscope Multiomic Amp 1 | Reagent | 30 MIN | 42°C |
| 76 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 77 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 78 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 79 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 80 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 81 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 82 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 83 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 84 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 85 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 86 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 87 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 88 | *Bond Wash Solution | Open Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 89 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 90 | RNAscope Multiomic Amp 2 | Reagent | 1 MIN | 42°C |
| 91 | RNAscope Multiomic Amp 2 | Reagent | 30 MIN | 42°C |
| 92 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 93 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 94 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 95 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 96 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 97 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 98 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 99 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 100 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 101 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 102 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 103 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 104 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 105 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 106 | RNAscope Multiomic Amp 3 | Reagent | 1 MIN | 42°C |
| 107 | RNAscope Multiomic Amp 3 | Reagent | 15 MIN | 42°C |
| 108 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 109 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 110 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 111 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 112 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 113 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 114 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 115 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 116 | RNAscope Multiomic HRP-C1 | Reagent | 1 MIN | 42°C |
| 117 | RNAscope Multiomic HRP-C1 | Reagent | 15 MIN | 42°C |
| 118 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 119 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 120 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 121 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 122 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 123 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 124 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 125 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 126 | RNAscope Multiomic TSA-F1 | Reagent | 1 MIN | Ambient |
| 127 | RNAscope Multiomic TSA-F1 | Reagent | 30 MIN | Ambient |
| 128 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 129 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 130 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 131 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 132 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 133 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 134 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 135 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 136 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 137 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 138 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 139 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 140 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 141 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 142 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 143 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 144 | RNAscope Multiomic LS HRP C2 | Reagent | 1 MIN | 42°C |
| 145 | RNAscope Multiomic LS HRP C2 | Reagent | 15 MIN | 42°C |
| 146 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 147 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 148 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 149 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 150 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 151 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 152 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 153 | RNAscope Multiomic TSA-F2 | Reagent | 1 MIN | Ambient |
| 154 | RNAscope Multiomic TSA-F2 | Reagent | 30 MIN | Ambient |
| 155 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 156 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 157 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 158 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 159 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 160 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 161 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 162 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 163 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 164 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 165 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 166 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 167 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 168 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 169 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 170 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 171 | RNAscope Multiomic LS HRP C3 | Reagent | 1 MIN | 42°C |
| 172 | RNAscope Multiomic LS HRP C3 | Reagent | 15 MIN | 42°C |
| 173 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 174 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 175 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 176 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 177 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 178 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 179 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 180 | RNAscope Multiomic TSA-F3 | Reagent | 1 MIN | Ambient |
| 181 | RNAscope Multiomic TSA-F3 | Reagent | 30 MIN | Ambient |
| 182 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 183 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 184 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 185 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 186 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 187 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 188 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 189 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 190 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 191 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 192 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 193 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 194 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 195 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 196 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 197 | RNAscope Multiomic LS HRP C4 | Reagent | 1 MIN | 42°C |
| 198 | RNAscope Multiomic LS HRP C4 | Reagent | 15 MIN | 42°C |
| 199 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 200 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 201 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 202 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 203 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 204 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 205 | RNAscope Multiomic TSA-F4 | Reagent | 1 MIN | Ambient |
| 206 | RNAscope Multiomic TSA-F4 | Reagent | 30 MIN | Ambient |
| 207 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 208 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 209 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 210 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 211 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 212 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 213 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 214 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 215 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 216 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 217 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 218 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 219 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 220 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 221 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 222 | RNAscope Multiomic LS HRP C5 | Reagent | 1 MIN | 42°C |
| 223 | RNAscope Multiomic LS HRP C5 | Reagent | 15 MIN | 42°C |
| 224 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 225 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 226 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 227 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 228 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 229 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 230 | RNAscope Multiomic TSA-F5 | Reagent | 1 MIN | Ambient |
| 231 | RNAscope Multiomic TSA-F5 | Reagent | 30 MIN | Ambient |
| 232 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 233 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 234 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 235 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 236 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 237 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 238 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 239 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 240 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 241 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 242 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 243 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 244 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 245 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 246 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 247 | RNAscope Multiomic LS HRP C6 | Reagent | 1 MIN | 42°C |
| 248 | RNAscope Multiomic LS HRP C6 | Reagent | 15 MIN | 42°C |
| 249 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 250 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 251 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 252 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 253 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 254 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 255 | TSA-DIG | Reagent | 1 MIN | Ambient |
| 256 | TSA-DIG | Reagent | 30 MIN | Ambient |
| 257 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 258 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 259 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 260 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 261 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 262 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 263 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 264 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 265 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 266 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 267 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 268 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 269 | Polaris 780 | Reagent | 1 MIN | Ambient |
| 270 | Polaris 780 | Reagent | 30 MIN | Ambient |
| 271 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 272 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 273 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 274 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 275 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 276 | DAPI | Reagent | 10 MIN | Ambient |
| 277 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 278 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 279 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

E

Appendix E. 2 secondary antibodies + 4 Primary conjugated antibodies

| | Reagent | Container name | Details (concentration, dilution) |
|---|--|------------------------|---|
| 1 | Salmon Sperm DNA | Open1 | 500 ug/mL, in multiomic antibody diluent |
| 2 | 10% NBF | 10% NBF | None |
| 3 | Primary Raw Antibody mix | CoDetection Antibody 1 | Multiomic antibody diluent |
| 4 | Secondary Conjugated Antibody mix | CoDetection Antibody 2 | Multiomic antibody diluent (See Appendix H for concentration) |
| 5 | Primary Conjugated Antibody mix | CoDetection Antibody 3 | Multiomic antibody diluent (See Appendix H for concentration) |
| 6 | Antibody Blocker (mouse and/or Rabbit IgG) | Antibody Blocker | 5ug/ml, in multiomic antibody diluent |

Slide Setup:

Slide properties ×

Pos 1

Tissue type: **Dispense volume:**

Test tissue 100 µL

Negative tissue 150 µL

Positive tissue

Staining mode:

Single Routine

Single

Process: IHC ISH

Marker: Mock Probe

Protocols

Staining: Primary Antibody + Secondary Antibody

Preparation: *Bake and Dewax

HIER: *ACD HIER 15 min with ER2 (95)

Enzyme: * - - -

Probe Application: *RNAscope 2.5 LSx Probe Application

Denaturation: * - - -

Hybridization: ACD 1 Min Hybridization

Probe Removal: *RNAscope 2.5 LSx Probe Removal

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-------------------------|-----------|-----------------|--------------|
| 1 | Open1 | Reagent | 0 MIN | Ambient |
| 2 | Open1 | Reagent | 60 MIN | Ambient |
| 3 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 4 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 5 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 6 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 7 | Co-Detection Antibody 1 | Reagent | 0 MIN | Ambient |
| 8 | Co-Detection Antibody 1 | Reagent | 60 MIN | Ambient |
| 9 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 10 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 11 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 12 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 13 | Co-Detection Antibody 2 | Reagent | 0 MIN | Ambient |
| 14 | Co-Detection Antibody 2 | Reagent | 30 MIN | Ambient |
| 15 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 16 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 17 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 18 | *Bond Wash Solution | Wash | 1MIN | Ambient |
| 19 | Antibody Blocker | Reagent | 0 MIN | Ambient |
| 20 | Antibody Blocker | Reagent | 30 MIN | Ambient |
| 21 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 22 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 23 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 24 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 25 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 26 | Co-Detection Antibody 3 | Reagent | 0 MIN | Ambient |
| 27 | Co-Detection Antibody 3 | Reagent | 60 MIN | Ambient |
| 28 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 29 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 30 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 31 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 32 | *Bond Wash Solution | Wash | 3MIN | Ambient |
| 33 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 34 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 35 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 36 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 37 | *Bond Wash Solution | Open Wash | 0 MIN | Ambient |
| 38 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 39 | 10% NBF | Reagent | 30 MIN | Ambient |
| 40 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 41 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 42 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 43 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 44 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 45 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 46 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 47 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 48 | PretreatPro | Reagent | 0 MIN | Ambient |
| 49 | PretreatPro | Reagent | 30 MIN | 40°C |
| 50 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 51 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 52 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 53 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 54 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 55 | Hydrogen Peroxide | Reagent | 10 MIN | Ambient |
| 56 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 57 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 58 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 59 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 60 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 61 | RNAscope Multiomic Amp 1 | Reagent | 1 MIN | 42°C |
| 62 | RNAscope Multiomic Amp 1 | Reagent | 30 MIN | 42°C |
| 63 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 64 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 65 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 66 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 67 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 68 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 69 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 70 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 71 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 72 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 73 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 74 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 75 | *Bond Wash Solution | Open Wash | 0 MIN | Ambient |
| 76 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 77 | RNAscope Multiomic Amp 2 | Reagent | 1 MIN | 42°C |
| 78 | RNAscope Multiomic Amp 2 | Reagent | 30 MIN | 42°C |
| 79 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 80 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 81 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 82 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 83 | *Bond Wash Solution | Wash | 3 MIN | Ambient |
| 84 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 85 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 86 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 87 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|-----------------------------|-----------|-----------------|--------------|
| 88 | RNAscope Multiomic LS Rinse | Reagent | 5 MIN | Ambient |
| 89 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 90 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 91 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 92 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 93 | RNAscope Multiomic Amp 3 | Reagent | 1 MIN | 42°C |
| 94 | RNAscope Multiomic Amp 3 | Reagent | 15 MIN | 42°C |
| 95 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 96 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 97 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 98 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 99 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 100 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 101 | *Bond Wash Solution | Open Wash | 1 MIN | Ambient |
| 102 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 103 | RNAscope Multiomic HRP-C1 | Reagent | 1 MIN | 42°C |
| 104 | RNAscope Multiomic HRP-C1 | Reagent | 15 MIN | 42°C |
| 105 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 106 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 107 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 108 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 109 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 110 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 111 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 112 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 113 | RNAscope Multiomic TSA-F1 | Reagent | 1 MIN | Ambient |
| 114 | RNAscope Multiomic TSA-F1 | Reagent | 30 MIN | Ambient |
| 115 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 116 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 117 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 118 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 119 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 120 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 121 | *Bond Wash Solution | Wash | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 122 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 123 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 124 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 125 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 126 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 127 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 128 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 129 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 130 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 131 | RNAscope Multiomic LS HRP C2 | Reagent | 1 MIN | 42°C |
| 132 | RNAscope Multiomic LS HRP C2 | Reagent | 15 MIN | 42°C |
| 133 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 134 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 135 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 136 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 137 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 138 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 139 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 140 | RNAscope Multiomic TSA-F2 | Reagent | 1 MIN | Ambient |
| 141 | RNAscope Multiomic TSA-F2 | Reagent | 30 MIN | Ambient |
| 142 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 143 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 144 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 145 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 146 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 147 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 148 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 149 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 150 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 151 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 152 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 153 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 154 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 155 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 156 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 157 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 158 | RNAscope Multiomic LS HRP C3 | Reagent | 1 MIN | 42°C |
| 159 | RNAscope Multiomic LS HRP C3 | Reagent | 15 MIN | 42°C |
| 160 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 161 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 162 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 163 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 164 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 165 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 166 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 167 | RNAscope Multiomic TSA-F3 | Reagent | 1 MIN | Ambient |
| 168 | RNAscope Multiomic TSA-F3 | Reagent | 30 MIN | Ambient |
| 169 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 170 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 171 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 172 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 173 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 174 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 175 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 176 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 177 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 178 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 179 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 180 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 181 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 182 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 183 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 184 | RNAscope Multiomic LS HRP C4 | Reagent | 1 MIN | 42°C |
| 185 | RNAscope Multiomic LS HRP C4 | Reagent | 15 MIN | 42°C |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 186 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 187 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 188 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 189 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 190 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 191 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 192 | RNAscope Multiomic TSA-F4 | Reagent | 1 MIN | Ambient |
| 193 | RNAscope Multiomic TSA-F4 | Reagent | 30 MIN | Ambient |
| 194 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 195 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 196 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 197 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 198 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 199 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 200 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 201 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 202 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 203 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 204 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 205 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 206 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 207 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 208 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 209 | RNAscope Multiomic LS HRP C5 | Reagent | 1 MIN | 42°C |
| 210 | RNAscope Multiomic LS HRP C5 | Reagent | 15 MIN | 42°C |
| 211 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 212 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 213 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 214 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 215 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 216 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 217 | RNAscope Multiomic TSA-F5 | Reagent | 1 MIN | Ambient |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|--------------------------------|-----------|-----------------|--------------|
| 218 | RNAscope Multiomic TSA-F5 | Reagent | 30 MIN | Ambient |
| 219 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 220 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 221 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 222 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 223 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 224 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 225 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 226 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 227 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |
| 228 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 229 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 230 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 231 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 232 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 233 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 234 | RNAscope Multiomic LS HRP C6 | Reagent | 1 MIN | 42°C |
| 235 | RNAscope Multiomic LS HRP C6 | Reagent | 15 MIN | 42°C |
| 236 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 237 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 238 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 239 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 240 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 241 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 242 | TSA-DIG | Reagent | 1 MIN | Ambient |
| 243 | TSA-DIG | Reagent | 30 MIN | Ambient |
| 244 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 245 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 246 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 247 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 248 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 249 | RNAscope Multiomic HRP Blocker | Reagent | 1 MIN | 42°C |
| 250 | RNAscope Multiomic HRP Blocker | Reagent | 15 MIN | 42°C |

| Step No. | Reagent | Step Type | Incubation Time | Temperature† |
|----------|---------------------|-----------|-----------------|--------------|
| 251 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 252 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 253 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 254 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 255 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 256 | Polaris 780 | Reagent | 1 MIN | Ambient |
| 257 | Polaris 780 | Reagent | 30 MIN | Ambient |
| 258 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 259 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 260 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 261 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 262 | *Bond Wash Solution | Wash | 1 MIN | Ambient |
| 263 | DAPI | Reagent | 10 MIN | Ambient |
| 264 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 265 | *Bond Wash Solution | Wash | 0 MIN | Ambient |
| 266 | *Bond Wash Solution | Wash | 0 MIN | Ambient |

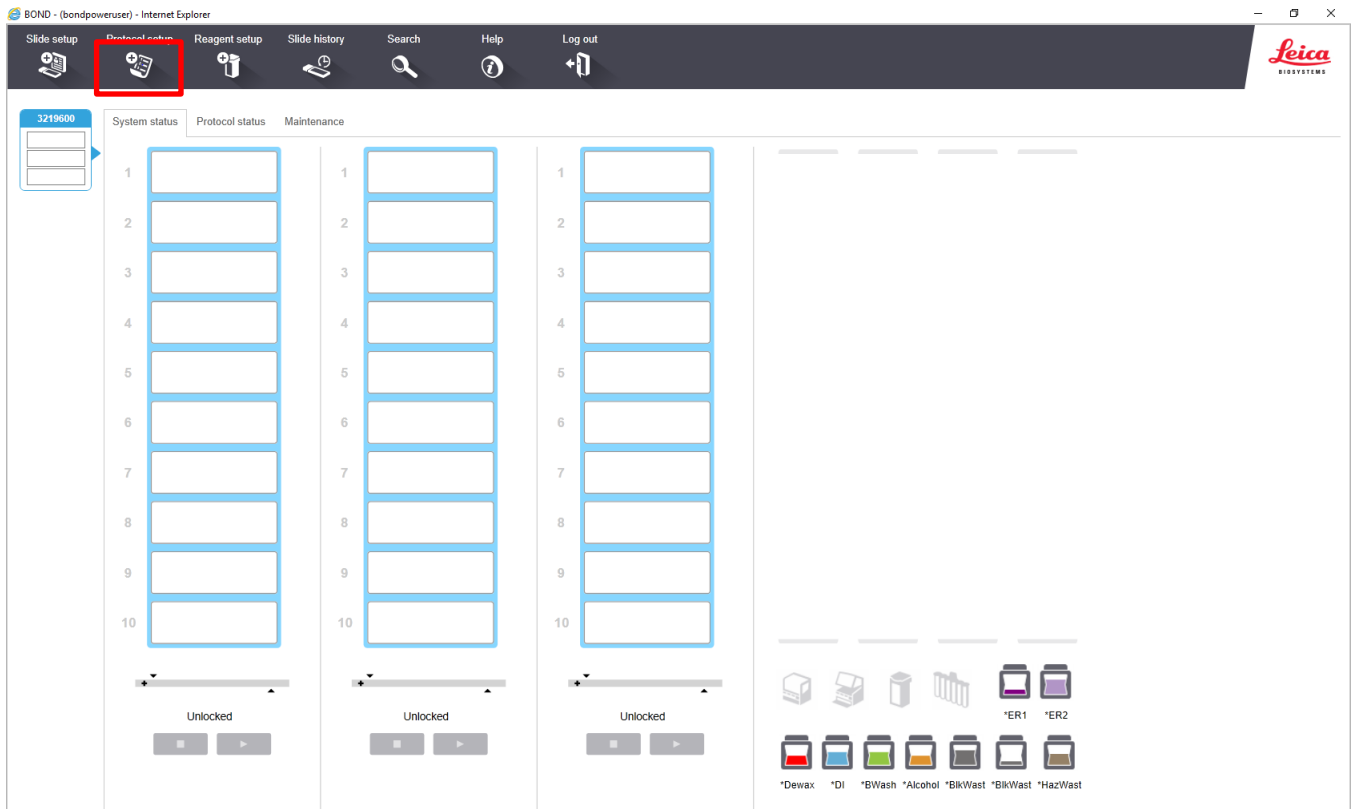
F

Appendix F. Edit the Epitope Retrieval Protocol

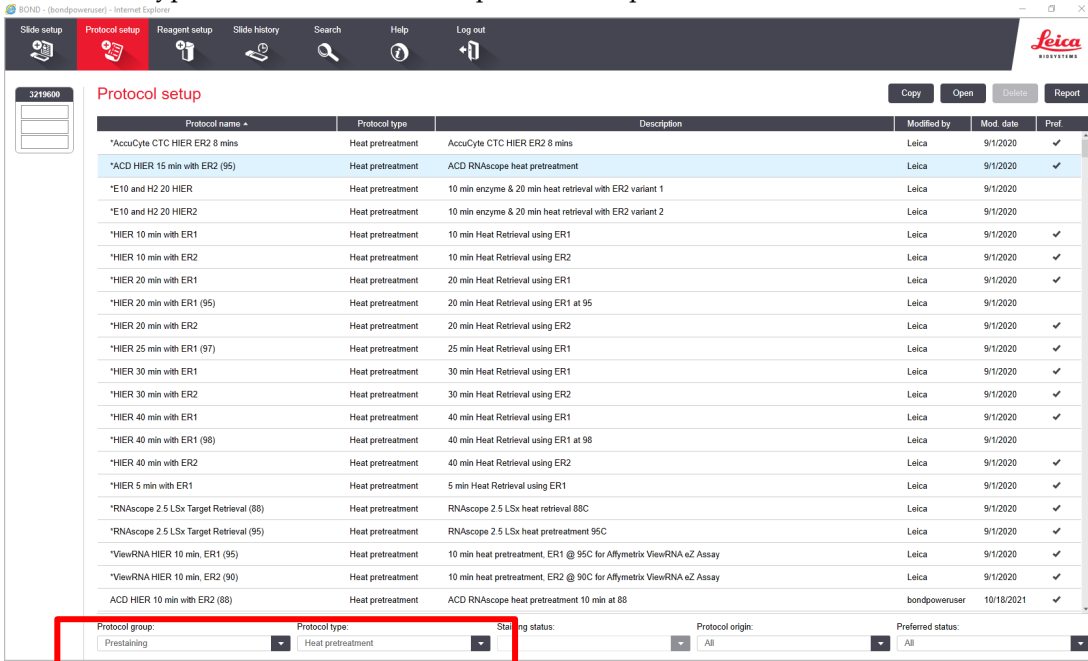
The following example shows how to edit the Epitope Retrieval procedure from within the software.

Create a prestaining protocol

1. Open the Leica BOND software and click on the **Protocol setup** icon as shown.



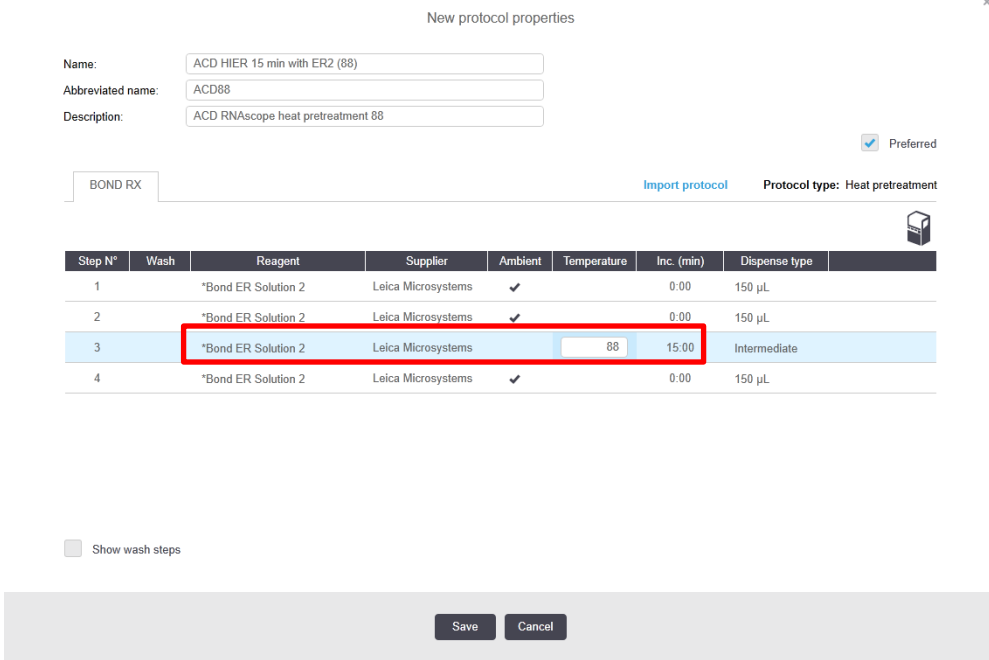
- Select **Prestaining** under the Protocol group menu and **Heat pretreatment** under the Protocol type menu to access the heat pretreatment protocols.



- Highlight the ***ACD HIER 15 min with ER2 (95)** protocol. Select **Copy**.

Note: ER2 = Epitope Retrieval 2.

- Rename the protocol as **ACD HIER 15 min with ER2 (88)**.
- Rename the Abbreviated name as **ER2-88**.
- Rename the Description to **ACD RNAscope heat pretreatment 88**.
- Highlight the third ***BOND ER Solution 2** step (see above) and change temperature to **88°C**.



8. For RNAscope, ER 2 temperature varies between 95°C and 88°C depending on the tissue type used. Please see Appendix A for a list of tissues.
9. Select **Save** to create a protocol for ER2 pretreatment at 88°C.
10. If needed, repeat Steps 1–8 to create new heating protocols for different incubation times (for example, ACD 25minER2).



Appendix G. Edit the Protease Protocol

The following example shows how to edit the Protease procedure from within the software.

1. Select **Enzyme Pretreatment** under the Protocol type menu (bottom left).
2. Highlight the ***ACD 15min Protease** protocol. Select **Copy**.

| Protocol name | Protocol type | Description | Modified by | Mod. date | Prof |
|--------------------------|---------------------|--|---------------|-----------|------|
| *ACD 15 min Protease | Enzyme pretreatment | ACD RNAscope enzyme pretreatment | Leica | 9/1/2020 | ✓ |
| *BaseScope LSx Enzyme | Enzyme pretreatment | 15 min Enzyme Pretreatment using BaseScope LSx Protease | Leica | 5/5/2021 | |
| *E10 and H2 20 | Enzyme pretreatment | 10 min enzyme & 20 min heat retrieval with ER2 | Leica | 9/1/2020 | |
| *Enzyme 1 (20) | Enzyme pretreatment | Enzyme 1 pretreatment (20 min) for ViewRNA eZ-L Assay | Leica | 9/1/2020 | |
| *Enzyme 1 for 10 min | Enzyme pretreatment | 10 min Enzyme Pretreatment using Enzyme 1 | Leica | 9/1/2020 | ✓ |
| *Enzyme 1 for 15 min | Enzyme pretreatment | 15 min Enzyme Pretreatment using Enzyme 1 | Leica | 9/1/2020 | ✓ |
| *Enzyme 1 for 5 min | Enzyme pretreatment | 5 min Enzyme Pretreatment using Enzyme 1 | Leica | 9/1/2020 | ✓ |
| *Enzyme 2 (20) | Enzyme pretreatment | Enzyme 2 pretreatment (20 min) for ViewRNA eZ-L Assay | Leica | 9/1/2020 | |
| *Enzyme 2 for 10 min | Enzyme pretreatment | 10 min Enzyme Pretreatment using Enzyme 2 | Leica | 9/1/2020 | ✓ |
| *Enzyme 2 for 15 min | Enzyme pretreatment | 15 min Enzyme Pretreatment using Enzyme 2 | Leica | 9/1/2020 | ✓ |
| *Enzyme 3 (20) | Enzyme pretreatment | Enzyme 3 pretreatment (20 min) for ViewRNA eZ-L Assay | Leica | 9/1/2020 | |
| *Enzyme 3 for 10 min | Enzyme pretreatment | 10 min Enzyme Pretreatment using Enzyme 3 | Leica | 9/1/2020 | ✓ |
| *Enzyme 3 for 15 min | Enzyme pretreatment | 15 min Enzyme Pretreatment using Enzyme 3 | Leica | 9/1/2020 | ✓ |
| *Enzyme 5 for 25 min | Enzyme pretreatment | 25 min Enzyme Pretreatment using Enzyme 5 | Leica | 9/1/2020 | ✓ |
| *Protease 20 min and fix | Enzyme pretreatment | Protease 20 min and fix | Leica | 9/1/2020 | ✓ |
| *RNAscope 2.5 LSx Enzyme | Enzyme pretreatment | 15 min Enzyme Pretreatment using RNAscope 2.5 LSx Protease | Leica | 9/1/2020 | ✓ |
| *ViewRNA Enzyme 1 (20) | Enzyme pretreatment | 20 min enzyme pretreatment for Affymetrix ViewRNA eZ Assay | Leica | 9/1/2020 | ✓ |
| *ViewRNA Enzyme 2 (20) | Enzyme pretreatment | 20 min enzyme pretreatment for Affymetrix ViewRNA eZ Assay | Leica | 9/1/2020 | ✓ |
| *ViewRNA Enzyme 3 (20) | Enzyme pretreatment | 20 min enzyme pretreatment for Affymetrix ViewRNA eZ Assay | Leica | 9/1/2020 | ✓ |
| 2hr 54C pretreat | Enzyme pretreatment | ACD RNAscope enzyme pretreatment 2hr 54C | bondpoweruser | 2/21/2023 | ✓ |
| 30min 40C pretreat | Enzyme pretreatment | ACD RNAscope enzyme pretreatment 30min 40c | bondpoweruser | 7/31/2023 | ✓ |

3. Rename the protocol to **ACD 25min Protease**.
4. Rename the Abbreviated name to **25minPro**.
5. Rename the Description to **ACD RNAscope 25min enzyme**.
2. Highlight the second ***ACD Enzyme** step. Keep the temperature at **40°C** and set the enzyme incubation time to the desired time (for example, 25min).

New protocol properties

x

Name:

Abbreviated name:

Description:

Preferred

BOND RX [Import protocol](#) Protocol type: Enzyme pretreatment

| Step N° | Wash | Reagent | Supplier | Ambient | Temperature | Inc. (min) | Dispense type |
|---------|------|-------------|---------------------------|-------------------------------------|-------------|------------|---------------|
| 2 | | *ACD Enzyme | Advanced Cell Diagnostics | | 40 | 0:00 | 150 µL |
| 3 | | *ACD Enzyme | Advanced Cell Diagnostics | | 40 | 25:00 | 50 µL |
| 7 | | *Open 0 Haz | User | <input checked="" type="checkbox"/> | | 10:00 | 150 µL |

Show wash steps

3. Select **Save**.
4. If needed, repeat Steps 1–7 to create a new protease protocol for different sample types (for example, ACD 10min Protease or ACD 15min Protease at ambient temperature).



Appendix H. Conjugated antibody concentration

The following guide shows how to dilute the conjugated antibodies with the Multiomic antibody diluent provided.

1. Determine the number of slides needed for the run.
2. Calculate total volume for primary and secondary conjugated antibodies. Make sure to add enough dead-volume to your calculation depending on the container type used. Use 6 mL BOND Titration containers for up to 10 slides.
3. 600 µL dead-volume when using a BOND Titration container (6mL)
4. Use the following table for suggested concentration of each conjugated antibody.
5. Dilute secondary conjugated antibody in a separate tube.
6. Pool all four primary conjugated antibodies together in same tube.
7. Add the diluted antibodies to the appropriate containers.
8. Assign fluorophores using the following recommendations for best results.

| RNAscope antibody | Cat No. | Channel | Dilution factor | Opal Dye | Dye Dilution |
|--------------------------|-----------|---------|-----------------|----------|---|
| RNAscope™ Ab Hs CD4-C3 | 322949 | C3 | 75x | 480 | 1:3000 |
| RNAscope™ Ab Hs CD8-C4 | 322951 | C4 | 75x | 690 | 1:5000 |
| RNAscope™ Ab Hs PanCK-C5 | 322952 | C5 | 75x | 780 | 1:500 (TSA-DIG) + 1:125 (Opal 780 Reagent†) |
| RNAscope™ Ab Hs FoxP3-C6 | 322953 | C6 | 75x | 520 | 1:5000 |
| RNAscope™ Ab NeuN-C3 | AB0018-C3 | C3 | 75x | 480 | 1:3000 |
| RNAscope™ Ab GFAP-C4 | AB0028-C4 | C4 | 75x | 520 | 1:3000 |
| RNAscope™ Ab IBA-1-C5 | AB0038-C5 | C5 | 75x | 690 | 1:5000 |
| RNAscope™ anti-rabbit-C1 | 322954 | C1 | 25x | 620 | 1:10000 |
| RNAscope™ anti-mouse-C2 | 322956 | C2 | 25x | 570 | 1:10000 |

I

Appendix I. Pretreatment Guidance for FFPE Samples – for RNA targets only

Follow the recommended pretreatment conditions based on your tissue type for:

- Any new or previously untested FFPE tissue types
- Samples prepared differently than the sample preparation protocol found in **Chapter 3**.
- For specific guidance on other sample preparations contact ACD Support at support.acd@bio-techne.com

Tissue-specific pretreatment conditions

Refer to the following table for tissue specific FFPE pretreatment conditions. For information about species or tissue type not listed here, contact support at support.acd@bio-techne.com.

| Species | Tissue Type | Pathology | Pretreatment Condition | Species | Tissue Type | Pathology | Pretreatment Condition |
|-----------|--------------------|-----------|------------------------|---------|---|-----------|------------------------|
| Mouse/Rat | Intestine | Normal | Standard | Human | Head | Cancer | Standard |
| | Intestine | Tumor | Standard | | Neck | Cancer | Standard |
| | Embryo | Normal | Standard | | Liver | Cancer | Standard |
| | Brain | Normal | Standard | | Liver | Normal | Standard |
| | Spleen | Normal | Standard | | Heart | Normal | Standard |
| | Eye/Retina | Normal | Extended | | GI tract | Normal | Standard |
| | Liver | Normal | Standard | | Kidney | Normal | Standard |
| | Kidney | Normal | Standard | | Skin | Normal | Standard |
| Human | Breast | Tumor | Standard | | Lymphoma | Cancer | Standard |
| | Colon | Tumor | Standard | | Thymus | Normal | Mild/Standard |
| | Colon | Normal | Standard | | Melanoma | Tumor | Standard |
| | Lung | Tumor | Standard | | Nevus | Benign | Standard |
| | Lung | Normal | Standard | | Placenta | Normal | Standard |
| | Prostate | Tumor | Standard | | Skin (TMA*) | Normal | Standard |
| | Prostate | Normal | Standard | | Breast (TMA*) | Normal | Standard |
| | Lymph node | Tumor | Standard | | Melanoma (TMA*) | Normal | Standard |
| | Lymph node | Normal | Mild | | Nevus (TMA) | Benign | Standard |
| | Tonsil | Normal | Mild/Standard | | Stomach (TMA) | Normal | Standard |
| | Pancreas | Normal | Standard | | Stomach (TMA) | Tumor | Standard |
| | Cervical | Cancer | Standard | | Cell pellets, fixed with 10% NBF | — | Mild |
| | Cervical | Normal | Standard | | HeLa or 3T3 cells, fixed with 10% Formaldehyde /PBS/ACD Control | — | Mild |
| | Cervical dysplasia | Abnormal | Standard | | Xenograft tissue | — | Mild |
| | Brain | Tumor | Standard | | | | |
| | Brain | Normal | Standard | | | | |

*Tissue Microarray

| Species | Tissue Type | Pathology | Pretreatment Condition | Species | Tissue Type | Pathology | Pretreatment Condition |
|----------------|----------------|-----------|------------------------|---------|----------------|---------------|------------------------|
| Cyno monkey | Spleen | Normal | Mild | Dog | Spleen | Normal | Mild |
| | Lymph Node | Normal | Mild | | Lymph Node | Mild | Mild |
| | Tonsil | Normal | Mild | | Tonsil | N.A. | N.A. |
| | Thymus | Normal | Mild | | Thymus | Mild | Mild |
| | Retina | Normal | Mild | | Retina | Mild | Mild |
| | Prostate Gland | Normal | Standard/Mild | | Prostate Gland | Mild | Mild |
| | Epididymis | Normal | Mild/Standard | | Epididymis | Mild | Mild |
| | Testis | Normal | Mild/Standard | | Testis | Mild/Standard | Mild/Standard |
| | Ovary | Normal | Mild/Standard | | Ovary | Mild/Standard | Mild/Standard |
| | Duodenum | Normal | Mild/Standard | | Duodenum | Normal | Mild |
| | Jejunum | Normal | Mild/Standard | | Jejunum | Normal | Mild |
| | Colon | Normal | Standard | | Colon | Normal | Mild |
| | Adrenal Gland | Normal | Mild/Standard | | Adrenal Gland | Normal | Standard/Mild |
| | Rat | Spleen | Normal | | Mild | | |
| Lymph Node | | Normal | Mild | | | | |
| Tonsil | | Normal | N.A. | | | | |
| Thymus | | Normal | Mild | | | | |
| Retina | | Normal | Mild | | | | |
| Prostate Gland | | Normal | Standard/Mild | | | | |
| Epididymis | | Normal | Standard | | | | |
| Testis | | Normal | Standard | | | | |
| Ovary | | Normal | Standard | | | | |
| Duodenum | | Normal | Standard/Mild | | | | |
| Jejunum | | Normal | Standard | | | | |
| Colon | | Normal | Standard | | | | |
| Adrenal Gland | | Normal | N.A. | | | | |



Appendix J. How to use LS PretreatPro – for RNA targets only

Prepare the instrument reagents

This workflow should be followed if you are performing a staining run with only RNA targets and want to utilize a protease-free permeabilization approach (see page 21 for decision tree). This workflow uses LS PretreatPro in place of traditional reagents.

| Traditional protease workflow | New protease free workflow |
|-------------------------------|----------------------------|
| LS Protease III | LS PretreatPro |

1. Place RNAscope 2.5 LS PretreatPro into a BOND Open container and register it as ***ACD Enzyme**.

Note: Visually identify the container as LS PretreatPro to avoid unintended use.

2. Place the ACD Amp Pro into a BOND Open container and register it as reagent created.

Note: Place only one *ACD Enzyme reagent on the instrument at a time.

3. When registering containers select the corresponding name of the reagent from the drop-down menu as showing in the following table under **Container Name**:

| Reagents | Container Name |
|-----------------------------------|-----------------------------|
| RNAscope 2.5 LS Hydrogen Peroxide | *Open 0 Haz |
| RNAscope 2.5 LS PretreatPro | *ACD Enzyme |
| RNAscope Multiomic LS AMP 1 | Amp 1 |
| RNAscope Multiomic LS AMP 2 | Amp 2 |
| RNAscope Multiomic LS AMP 3 | Amp 3 |
| RNAscope Multiomic LS HRP C1 | MOHRPC1 |
| RNAscope Multiomic LS HRP C2 | MOHRPC2 |
| RNAscope Multiomic LS HRP C3 | MOHRPC3 |
| RNAscope Multiomic LS HRP C4 | MOHRPC4 |
| ACD Multiomic TSA-F1 | MOTSAF1 |
| ACD Multiomic TSA-F2 | MOTSAF2 |
| ACD Multiomic TSA-F3 | MOTSAF3 |
| ACD Multiomic TSA-F4 | MOTSAF4 |
| RNAscope 2.5 LS Rinse | RNAscope Multiomic LS Rinse |
| RNAscope 2.5 LS Target Probe | Variable (probe 1 2.5) |

* Indicates reagent is hard coded in software by Leica Biosystems.

Note: When paired with correlated Enzyme treatment protocol, container names *Open 0 Haz and *RNAscope 2.5 LSx Hydrogen Peroxide are equivalent and *ACD Enzyme and *RNAscope 2.5 LSx Enzyme are equivalent.

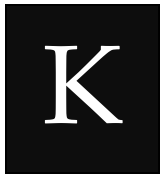
5. Enter the RNAscope 2.5 LS Reagent Kit lot number and the expiration date in their respective fields. Select **OK**.

IMPORTANT! Do not introduce bubbles into the solutions by shaking the containers. To mix reagents, gently invert the containers several times. If bubbles are present, leave the containers out at room temperature until the bubbles dissipate.

Update the protocol

1. Filter **Protocol Group** by **Prestaining**, and filter for **Protocol type** by **Enzyme Pretreatment**. Find existing protocol ***ACD 15min Protease** that includes the two reagents *ACD Enzyme and *Open 0 Haz.
2. Copy and create a new protocol:
 - a. Change the *ACD Enzyme incubation time to **15 MIN** per step for two steps and the temperature to **40°C**.
 - b. Keep ***Open 0 Haz** incubation time at **10 MIN** and temperature at **Ambient**. Save the protocol with a new name such as **ACD 30min PretreatPro**.
3. Refer to **Appendix C** for detailed instructions on editing an Enzyme treatment protocol.
4. Run all the other parts of the assay as usual and change only this reagent.
5. The final Slide setup for an FFPE sample should look like the following considering proper HIER condition choice.
 - a. **Staining:** Choose an edited LS multiplex/multiomics staining protocol as created in Chapter 6.
 - b. **Preparation:** Select ***Bake and Dewax**.
 - c. **HIER:** Choose ***ACD HIER 15 min with ER2 (95)**.
 - d. **Enzyme:** Choose the protocol created in step 2.
 - e. **Probe Application:** Select ***RNAscope 2.5 LSx Probe Application**.
 - f. **Denaturation:** Select ***...**
 - g. **Hybridization:** Select ***RNAscope 2.5 LSx Hybridization**.
 - h. **Probe Removal:** Select ***RNAscope 2.5 LSx Probe Removal**.
 - i. Select **Add slide**.
6. After adding all the slides to the study, select **Close** to return to the Slide setup screen.
7. Proceed to **Chapter 7. Run the RNAscope 2.5 LS Assay**.

Note: For all protease-free workflows using LS PretreatPro and involving a low RNA expressor and/or a dense tissue like liver or spleen, we recommend boosting the ISH signal by increasing the target retrieval (ER2) strength [duration, temperature, or volume] or increasing fluorophore concentration to achieve preferred results.



Appendix K. Slide Setup for Additional Tissue Types – for RNA targets only

Alternatively prepared samples can be stained on the BOND RX using the following slide setup parameters.

Note: Choose appropriate staining and hybridization related protocols depending on whether you are using the mock probe workflow (Chapter 5) or the standard probe workflow (Chapter 6).

Fixed-frozen tissues

As described in **Chapter 4**, these tissues need a gentle target retrieval step.

1. In Slide setup, select the following:
 - a. Staining: Choose the appropriate protocol for the chemistry and workflow you are using.
 - b. Preparation: Select *----.
 - c. HIER: Choose ***ACD HIER 5 min with ER2 (95)**. See **Appendix B** to create this protocol.
 - d. Enzyme: Select the appropriate protocol for the chemistry and workflow you are using; ***ACD 15min Protease** or **RNAscope LSx Enzyme**.
 - e. Probe Application: Select ***RNAscope 2.5 LSx Probe Application**.
 - f. Denaturation: Choose *....
 - g. Hybridization: Choose the appropriate protocol for the chemistry and workflow you are using; **ACD 1 min Hybridization** or ***RNAscope 2.5 LSx Hybridization**.
 - h. Probe Removal: Select ***RNAscope 2.5 LSx Probe Removal**.
2. Protease incubation time may need to be adjusted but start with **15 MINS** as that works for most tissues.

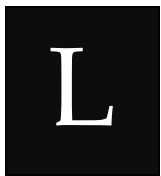
Fresh-frozen tissues

As described in **Chapter 4**, these tissues do NOT need a target retrieval. Instead, permeabilize the tissue at ambient temperature with a stronger protease such as RNAscope LS Protease IV (Cat. No. 322140).

1. In Slide setup, please skip the following steps: 1) Bake or Bake and Dewax 2) Heat retrieval. Choose the following instead:
 - a. Staining: Select the appropriate protocol for the chemistry and workflow you are using.
 - b. Preparation: Choose *----.
 - c. HIER: Choose *----.
 - d. Enzyme: Select **ACD 30min RT with LS Protease IV[†]**.
 - e. Probe Application: Select ***RNAscope 2.5 LSx Probe Application**.
 - f. Denaturation: Select *....

- g. Hybridization: Choose the appropriate protocol for the chemistry and workflow you are using: **ACD 1 min Hybridization** or ***RNAscope 2.5 LSx Hybridization**.
- h. Probe Removal: Select ***RNAscope 2.5 LSx Probe Removal**.
 - †See **Appendix G** to edit the protease protocol.

Note: Start your run immediately after setting it up. Do not use a delayed start. This causes poor protease spreadability and negatively impacts results. When the run is complete, the BOND RX rinses the slides every 10 minutes which can impact the counterstain. Set up the instrument as late in the day as possible. Rinsing does not affect the RNAscope signal and counterstaining can be repeated offline in the morning if needed.




Appendix L. Safety

Chemical safety

 **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, visit <http://www.acdbio.com/technical-support/user-manuals>.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

 **WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at www.cdc.gov/biosafety
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials
- Additional information about biohazard guidelines is available at www.cdc.gov/

In the EU:

- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition
- Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available at: <https://acdbio.com/technical-support/user-manuals>. For the SDSs of chemicals not distributed by Advanced Cell Diagnostics, contact the chemical manufacturer.

Obtaining support

For the latest services and support information, go to: <https://acdbio.com/technical-support/support-overview>.

At the website, you can:

- Access telephone and fax numbers to contact Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Search for user documents, SDSs, application notes, citations, training videos, and other product support documents.
- Find out information about customer training events.

Contact information

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 Newark, CA 94560
 Toll Free: 1-877-576-3636
 Direct: 1-510-576-8800
 Fax: 1-510-576-8801
 Information: info.acd@bio-techne.com
 Orders: orders.acd@bio-techne.com
 Support Email: support.acd@bio-techne.com

Limited product warranty

Advanced Cell Diagnostics, Inc. and/or its affiliate(s) warrant their products as set forth in the ACD General Terms and Conditions of Sale found on the ACD website. If you have any questions, please contact Advanced Cell Diagnostics at: <https://acdbio.com/about/contact>.

Headquarters

7707 Gateway Blvd Suite 200, Newark, CA 94560
Phone 1-510-576-8800 Toll Free 1-877-576-3636

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