

# Ordering Checklist for RNAscope™ Multiomic LS Assay for Leica Biosystems BOND RX

Note: this checklist does not include materials needed for the preparation of the tissue blocks (sample fixation, embedding, sectioning). Tissue should be sectioned on Superfrost Plus slides. For complete instructions, see the user manual. For questions or support, contact your ACD representative at +1 (877) 576-3636.

## ASSAY DESIGN OVERVIEW // 01

Use this table to plan the channels needed for your assay based on your desired targets. Note that antibodies are available in specific channels. Select individual RNAscope primary antibodies, which do not require secondaries, from available pre-configured panels. Probes can be designed to any open channel.

Target	RNAscope channel					
	C1	C2	C3	C4	C5	C6
<b>Protein targets with your primaries and RNAscope secondaries.</b>	Your rabbit primary + RNAscope anti-rabbit-secondary	Your mouse primary + RNAscope anti-mouse-secondary	—	—	—	—
<b>Protein targets selected from RNAscope primary antibodies. No secondaries necessary.</b>	—	—	RNAscope primary C3:_____	RNAscope primary C4:_____	RNAscope primary C5:_____	RNAscope primary C6:_____
<b>RNA targets using RNAscope probes designed to any open channel.</b>	RNAscope probe C1:_____	RNAscope probe C2:_____	RNAscope probe C3:_____	RNAscope probe C4:_____	RNAscope probe C5:_____	RNAscope probe C6:_____

## Order Assay Reagents

Assay Reagents	ACD Catalog number
<input type="checkbox"/> <b>RNAscope Multiomic CORE &amp; C1-C6 Channel Kit</b> <i>Suitable for detection of up to 6 targets (RNA and/or protein targets)</i>	323175 (60 slides) 323465 (20 slides)
<input type="checkbox"/> <b>RNAscope Multiomic CORE &amp; C1-C3 Channel Kit</b> <i>Suitable for detection of up to 3 targets (RNA and/or protein targets).</i>	323155 (60 slides) 323460 (20 slides)
<b>Individual C4-C6 channel items add additional channel capability to the CORE &amp; C1-C3 channel kit.</b>	
<b>RNAscope Multiomic C4 Channel Reagents</b>	322950 (60 slides) 323445 (20 slides)
<b>RNAscope Multiomic C5 Channel Reagents</b>	322955 (60 slides) 323450 (20 slides)
<b>RNAscope Multiomic C6 Channel Reagents</b>	322960 (60 slides) 323455 (20 slides)
<b>Special configurations for the RNAscope Protein Proximity assay</b>	
<input type="checkbox"/> <b>RNAscope™ Multiomic LS Protein Proximity Detection Kit-C6</b> <i>Includes the CORE and C6 channel reagents. For use with the Proximity antibodies. Enables detection of the proximity signal alone without other targets.</i>	322411 (60 slides) 322409 (20 slides)

## Order Antibodies and Probes

### User Provided Primary Antibodies

Order user provided rabbit or mouse antibodies if using RNAscope Secondary Antibodies or performing the RNAscope Protein Proximity assay. Note that the Proximity assay requires both a rabbit and a mouse hosted primary.

Item
<input type="checkbox"/> <b>User provided primary antibody, rabbit host</b>
<input type="checkbox"/> <b>User provided primary antibody, mouse host</b>

### RNAscope Secondary Antibodies

Only required if detecting proteins with user provided primary antibodies. Note that secondary antibodies are available only in the listed channels.

Item	ACD Catalog number (60 slides)
<input type="checkbox"/> <b>RNAscope anti-rabbit-C1</b>	322954
<input type="checkbox"/> <b>RNAscope anti-mouse-C2</b>	322956

## RNAscope Primary Antibody Panels

Optional, no secondary antibodies required. Note that panel antibodies are available only in the listed channels.

RNAscope Human Tumor Infiltrating Lymphocyte (TIL) Antibodies	ACD Catalog number (20 slides)
<input type="checkbox"/> RNAscope Ab Hs CD4-C3	322949
<input type="checkbox"/> RNAscope Ab Hs CD8-C4	322951
<input type="checkbox"/> RNAscope Ab Hs PanCK-C5	322952
<input type="checkbox"/> RNAscope Ab Hs FoxP3-C6	322953
<input type="checkbox"/> <b>Human Tumor Infiltrating Lymphocyte (TIL) Panel Antibody Pack</b> <i>Includes all four human TIL panel antibodies</i>	323185

RNAscope Neural Antibodies <i>Cross-reacts with mouse, human, and non-human primate proteins</i>	ACD Catalog number (20 slides)
<input type="checkbox"/> RNAscope Ab NeuN-C3	AB0014-C3
<input type="checkbox"/> RNAscope Ab GFAP-C4	AB0024-C4
<input type="checkbox"/> RNAscope Ab IBA-1-C5	AB0034-C5
<input type="checkbox"/> <b>RNAscope Multiomic™ Neural antibody Panel Pack</b> <i>Includes all three neuronal primary antibodies</i>	321780

## RNAscope Protein Proximity Assay Antibodies

Required for performing the RNAscope Protein proximity assay.

Item	ACD Catalog number (20 slides)
<input type="checkbox"/> <b>RNAscope™ Protein Proximity secondary antibodies-rabbit and mouse</b> <i>Designed for the C6 channel. Requires user provided rabbit and mouse primary antibodies.</i>	322415
<input type="checkbox"/> <b>RNAscope™ ProteinProximity antibodies-Human PD1/PD-L1</b> <i>Designed for the C6 channel. Does not require use of secondary antibodies.</i>	322405

## RNA Target Probes

Order probes in appropriate channels. Probes can be selected in any open channel.

Probes	ACD Catalog number
<b>RNAscope LS Target Probes</b>	
<input type="checkbox"/> RNAscope LS Target Probes – [species] – [gene] -C1	
<input type="checkbox"/> RNAscope LS Target Probes – [species] – [gene] -C2	
<input type="checkbox"/> RNAscope LS Target Probes – [species] – [gene] -C3	
<input type="checkbox"/> RNAscope LS Target Probes – [species] – [gene] -C4	
<input type="checkbox"/> RNAscope LS Target Probes – [species] – [gene] -C5	
<input type="checkbox"/> RNAscope LS Target Probes – [species] – [gene] -C6	
<input type="checkbox"/> RNAscope Multiomic LS 6-plex Positive Control Probes- Hs <i>Control probes to other species also available.</i>	323198
<input type="checkbox"/> RNAscope Multiomic LS 6-plex Negative Control Probes	323208
<input type="checkbox"/> RNAscope 2.5 LS Blank Probe diluent — required if not using a C1 probe <i>Included if using a C1 RNAscope probe but required as a separate item if not using a C1 probe. Approximately 500uL per probe per slide is required, plus container dead volume.</i>	300048

## Order TSA Fluorophores

If using Vivid TSA Fluorophores for 3-plex assays

Item	ACD Catalog number	3-plex
<input type="checkbox"/> TSA Vivid Fluorophore 520	323271	✓
<input type="checkbox"/> TSA Vivid Fluorophore 570	323272	✓
<input type="checkbox"/> TSA Vivid Fluorophore 650	323273	✓

## If using Akoya Biosciences TSA Fluorophores for assays up to 6-plex

Item	Akoya Biosciences Catalog number	3-plex	4-plex	5 or 6-plex
<input type="checkbox"/> Opal 480 Reagent Pack	FP1500001KT			✓
<input type="checkbox"/> Opal 520 Reagent Pack	FP1487001KT	✓	✓	✓
<input type="checkbox"/> Opal 570 Reagent Pack	FP1488001KT	✓	✓	✓
<input type="checkbox"/> Opal 620 Reagent Pack	FP1495001KT			✓
<input type="checkbox"/> Opal 690 Reagent Pack	FP1497001KT	✓	✓	✓
<input type="checkbox"/> Opal Polaris 780 Reagent Pack	FP1501001KT			
<input type="checkbox"/> Antibody Diluent/Block (needed when using Opal Polaris 780)	ARD1001EA		✓	✓

Note: For 5-plex assay, select any 5 of the recommended fluorophores to match your imaging capability.

## Order Materials from Leica Biosystems

### Instrument/Software

- Leica Biosystems' BOND RX System — automated slide stainer
- Software Version 6.0 or greater

### Consumables

Item	Notes	Leica Biosciences Catalog number
<input type="checkbox"/> BOND 30 mL Open containers	QTY ~2 packs of 10 (including those on Research Detection System)	OP309700
<input type="checkbox"/> BOND 6 mL Titration kit (10 pack)	QTY 2 packs; 14 needed for training; 1/fluorophore except 780	OPT9049
<input type="checkbox"/> BOND Research Detection System	New and unregistered detection system required	DS9455/DS9777
<input type="checkbox"/> BOND Universal Cover tile		S21.4611
<input type="checkbox"/> BOND Epitope Retrieval Solution 1-1L (RTU)	Not used, but may need on deck depending on software version	AR9961
<input type="checkbox"/> BOND Epitope Retrieval Solution 2-1L (RTU)	Used for HIER protocols	AR9640
<input type="checkbox"/> BOND Dewax Solution – 1L (RTU)		AR9222
<input type="checkbox"/> BOND Wash Solution 10X Concentrate – 1L		AR9590
<input type="checkbox"/> BOND Aspirating Probe Cleaning System		CS9100
<input type="checkbox"/> BOND Mixing Stations		S21.1971

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## Other Required Materials

Item	Vendor	Catalog number
<input type="checkbox"/> Salmon Sperm DNA, sheared (10mg/ml)	ThermoFisher	AM9680
<input type="checkbox"/> Neutral Buffered Formalin solution, 10%	Sigma-Aldrich	HT5012-1CS
<input type="checkbox"/> Prolong Gold Antifade Mountant	ThermoFisher	P36930
<input type="checkbox"/> Mouse IgG2A Isotype Control	R&D Systems	MAB003
<input type="checkbox"/> Normal Rabbit IgG Control	R&D Systems	MAB1050

## Kit Components and Storage

Kit/Box	Component	60-slide kit Volume/Amount	20-slide kit Volume/Amount	Storage Temp
<b>RNAscope Multiomic LS CORE Reagents</b>  <b>Cat No.:</b> <b>60-slide kit: 322930</b> <b>20-slide kit: 323425</b>	RNAscope Multiomic LS AMP 1	21 mL x 1 bottle	8 mL x 1 bottle	consult product label
	RNAscope Multiomic LS AMP 2	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic LS AMP 3	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic LS Hydrogen Peroxide	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic LS Rinse	29 mL x 3 bottles	11 mL x 2 bottle	
	RNAscope Multiomic LS DAPI	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope 2.5 LS Protease III	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope PretreatPro™	28mL x 1 bottle	8 mL x 1 bottle	
<b>RNAscope Multiomic Channel Reagents, C1-C6</b>  <b>Cat Nos.:</b> <b>60-slide kit:</b> <b>322935, 322940,</b> <b>322945, 322950,</b> <b>322955, 322960</b>  <b>20-slide kit:</b> <b>323430, 323435,</b> <b>323440, 323445,</b> <b>323450, 323455</b>	RNAscope Multiomic LS HRP	21 mL x 1 bottle	8 mL x 1 bottle	consult product label
	RNAscope Multiomic TSA Buffer	29 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic HRP Blocker	29 mL x 1 bottle	8 mL x 1 bottle	
RNAscope antibodies	—	—	—	consult product label

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