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# AUTOMATED ASSAY: RNAscope® LS Multiplex Fluorescent Assay on Leica Biosystems' BOND RX System

Doc. No. 47-006-CKL

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## Reagents/Materials required from Leica Biosystems

- BOND Research Detection System (Cat. No. DS9455).
- BOND Open Containers (Cat. No. Op309700). Fill these containers with reagents from the RNAscope LS Multiplex Fluorescent Assay.
- BOND Universal Covertiles (Cat. No. S21.2001).
- BOND Dewax Solution (Cat. No. AR9222). Place solution in one of the large containers located in the bottom of the instrument.
- BOND Epitope Retrieval Solution 1 and 2 (Cat. Nos. AR9961 and AR9640).
- BOND Wash Solution 10X (Cat. No. AR9590). Dilute solution 1:10 and place in one of the large containers located in the bottom of the instrument.
- BOND Aspirating Probe Cleaning System (Cat. No. CS9100).
- BOND Mixing Stations (Cat. No. S21.1971). Replace every month.



## Reagents/Materials required from ACD for RNAscope

- RNAscope LS Multiplex Fluorescent Reagent Kit (Cat. No. 322800). The kit contains all the detection and pretreatment reagents needed to run the RNAscope assay on the Leica BOND RX, except for the RNA-specific probes.
- RNAscope 3-plex LS Multiplex Positive Control Probe (Cat. No. 320868 for Human POLR2A-C1/PPIB-C2/UBC-C3; Cat No. 320888 for Mouse POLR2A-C1/PPIB-C2/UBC-C3; Cat. No. 320838 for HeLa Cells TBP-C1/PPIB-C2/POLR2A-C3). Probes target common housekeeping genes to help qualify samples and interpret results.
- RNAscope 3-plex LS Multiplex Negative Control Probe (Cat. No. 320878). The probe targets the bacterial dapB gene in three channels to control for background noise, and to help interpret assay results (DapB-C1/DapB-C2/DapB-C3).

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- RNAscope 2.5 LS Target Probes target specific RNAs of interest in Channel C1, Channel C2, Channel C3, and Channel C4.
  - Optional RNAscope Control Slides (Cat. No. 310045 for Human Cell Pellet; Cat. No. 310023 for Mouse 3T3 Cell Pellet).
  - Fluorescent Microscope (eg. Leica DM Series, Zeiss Axio Imager). We recommend using a multispectral fluorescent imaging system for tissue with high auto-fluorescence.



### **Additional Reagents/Materials required from ACD for the RNAscope 4-plex assay**

- RNAscope LS 4-Plex Ancillary Kit (Cat. No. 323120). The kit is designed to work with the RNAscope LS Multiplex Fluorescent Reagent Kit (Cat. No. 322800), and C1, C2, C3, and C4 Channel Target Probes for 4-plex fluorescent *in situ* hybridization. HRP-C4, and HRP blocker are included.
- Species specific RNAscope 4-plex Positive Control Probes for RNAscope Multiplex Fluorescent Assay- POLR2A (Channel C1), PPIB (Channel C2), UBC (Channel C3), and HPRT1 (Channel C4) UBC has the highest relative expression level; PPIB is considered to be a moderate-high expressor, while HPRT1 and POLR2A are moderate-to low expressor targets.
- RNAscope 4-plex LS Multiplex Negative Control Probe (Cat. No. 321838).



### **Reagents/Materials provided by Researcher**

- TSA<sup>®</sup> Plus fluorescein System; recommended dilution range 1:750 - 1:3000 (Part No. NEL741001KT and NEL741001KTK; Perkin Elmer).
- TSA Plus Cyanine 3 System; recommended dilution range 1:750 – 1:3000 (Part No. NEL744001KT and NEL744B001KT; Perkin Elmer).
- TSA Plus Cyanine 5 System; recommended dilution range 1:750 – 1:3000 (Part No. NEL745001KT and NEL745B001KT; Perkin Elmer).
- For 4-plex assays, we recommend the following dyes at a dilution range of 1:750 – 1:3000:
  - Opal 520 Reagent Pack (Part No. FP1487001KT)
  - Opal 570 Reagent Pack (Part No. FP1488001KT)
  - Opal 620 Reagent Pack (Part No. FP1495001KT)
  - Opal 690 Reagent Pack (Part No. FP1497001KTP)

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**Note:** Always refer to the latest version of the user manuals for the complete set of materials required to run the RNAscope assay, available at <http://www.acdbio.com/technical-support/user-manuals>.

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