RNAscope® LS Multiplex Fluorescent Assay on Leica Biosystems' BOND RX System

Doc. No. CHK 47-006

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 help interpret assay results (*dapB*-C1/*dapB*-C2/*dapB*-C3).

☐ 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 Flourescent 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® Plus fluorescein System; recommended dilution range 1:750 - 1:3000 (Part No. NEL741001KT; Akoya Biosciences).

☐ TSA® Plus Cyanine 3 System; recommended dilution range 1:750 – 1:3000 (Part No. NEL744001KT; Akoya Biosciences).

☐ TSA® Plus Cyanine 5 System; recommended dilution range 1:750 – 1:3000 (Part No. NEL745001KT; Akoya Biosciences).

☐ 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)

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.