CHECKLIST

MANUAL ASSAY: RNAscope® Multiplex Fluorescent Assay v2

Doc. No. 47-007-CKL

Reagents/Materials required from ACD

- ☐ RNAscope Target Probes in Channel C1, Channel C2, and Channel C3 (C4 Channel probes are required to perform the 4-plex assay).
- ☐ Species specific RNAscope 3-plex Positive Control Probes- POLR2A (Channel C1), PPIB (Channel C2), UBC (Channel C3), and HPRT1 (Channel C4). Probes target common housekeeping genes to help qualify samples and interpret results.
- ☐ RNAscope 3-plex Negative Control Probe (Cat. No. 320871). The probe targets the bacterial dapB gene to control for background noise, and to help interpret assay results.
- ☐ RNAscope Multiplex Fluorescent Reagent Kit v2 (Cat. No. 323100). The kit provides enough reagents to stain ~20 tissue sections, each with an area of approximately 20 mm x 20 mm (0.75in x 0.75in). The reagent kit contains pretreatment kit, detection kit, TSA buffer and wash buffer.
- ☐ HybEZ™ Hybridization System with ACD EZ-Batch Slide System I or II (Cat. Nos. 321461 and 321462; Cat Nos. 321711 and 321721). The system maintains optimum humidity and temperature at 40°C during RNAscope hybridization.
- ☐ RNAscope Control Slides (Cat. No. 310045 for Human Cell Pellet; Cat. No. 310023 for Mouse 3T3 Cell Pellet).
- ☐ ImmEdge™ Hydrophobic Barrier Pen (Cat. No. 310018) is the only pen that will maintain a hydrophobic barrier throughout the RNAscope procedure. Do not use any other pen.

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

- ☐ RNAscope 4-Plex Ancillary Kit for Multiplex Fluorescent Kit v2 (Cat. No. 323120). The kit is designed to work with the RNAscope Multiplex Fluorescent Reagent Kit v2 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 Multiplex Negative Control Probe Probe (Cat. No. 321831).

Reagents/Materials provided by Researcher

☐ Superfrost® Plus slides (Fisher Scientific) are required. Other slide types may result in tissue detachment.

☐ ProLong® Gold Antifade Reagent Life Technologies (P36930).

☐ Fresh reagents (e.g. ethanol and xylene).

☐ 20X SSC (final concentration needed is 5X SSC).

☐ Fresh 10% NBF (neutral-buffered formalin).

☐ Hotplate, drying oven, water bath, thermometer, and microscope.

☐ Paraffin wax, microtome, and fume hood.

☐ TSA® 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. FP1497001KT)

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.