

Manual assay: RNAscope® Multiplex Fluorescent Assay v2

Doc. No. CHK 47-007



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: Human Cat.No.320861; Mouse Cat.No.320881; Rat CatNo.320891). 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 (Cat. Nos. 321461, 110VAC and 321462, 220VAC) or II (Cat. Nos. 321711, 110VAC and 321721, 220VAC). 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 as the barrier may not stay intact throughout the workflow).



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. It contains C4 HRP and HRP blocker.
- 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 (Human Cat.No.321801; Mouse Cat.No.321818; Rat CatNo.321821).

- RNAscope® 4-plex Multiplex Negative Control Probe (Cat. No. 321831). The probe targets the bacterial *dapB* gene to control for background noise, and help interpret assay results.



Reagents/Materials provided by Researcher

- Superfrost® Plus slides (Fisher Scientific Part No.12-550-15) are required. Other slide types may result in tissue detachment.
- ProLong® Gold Antifade Reagent (Life Technologies, Part No. 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.
- Purchased from Akoya Biosciences. For 3-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 690 Reagent Pack (Part No. FP1497001KT)
- Purchased from 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. 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>.