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# Checklist for Manual RNAscope® 2.5 Multiplex Fluorescent Assay

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Doc. No. CHK 47-005



## Reagents/Materials required from ACD

- RNAscope Target Probes in Channel C1, Channel C2, and Channel C3.
- 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 Fluorescent Reagent Kit (Cat. No. 320850). The kit contains a pretreatment kit for fresh frozen samples, a detection kit, and wash buffer. The pretreatment kit can be used for fresh frozen samples.
- 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.
- RNAscope Probe Diluent (Cat. No. 300041); only required if using the C2 and C3 probes.



## Reagents/Materials provided by Researcher

- Superfrost® Plus slides (Fisher Scientific) are required. Other slide types may result in tissue detachment.
- ProLong® Gold Antifade Reagent from Life Technologies (P36930).
- Fresh reagents (e.g. ethanol and 1X PBS).

- Fresh 10% NBF (neutral-buffered formalin).
- Hotplate, water bath, thermometer, and microscope.
- Cryostat and fume hood.

**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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