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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) (Human Cat.No.320861; Mouse Cat.No.320881; Rat Cat.No.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® Fluorescent Multiplex 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 or fixed frozen samples. Use of this chemistry with FFPE samples is not recommended. Contact your sales or support representative for additional guidance.
- 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).
- RNAscope® Probe Diluent (Cat. No. 300041); only required if using the C2 and C3 probes alone without a channel 1 probe.



## 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>.