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# Checklist for Manual RNAscope® 2.5 Duplex Assay

Doc. No. CHK 47-004

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## Reagents/Materials required from ACD

- RNAscope Target Probes in Channel C1 and Channel C2.
- RNAscope 2.5 Duplex Positive Control Probe (PIIB-C1 / POLR2A-C2) Cat No. 321641 for Human and Cat No. 321651 for Mouse, targeting common housekeeping genes to help qualify samples and interpret assay results.
- RNAscope 2-plex Negative Control Probe (Cat. No. 320751). The probe targets the bacterial dapB gene to control for background noise, and to help interpret assay results.
- RNAscope 2.5 Duplex Reagent Kit Cat. No. 322430 consisting of a pretreatment kit (for FFPE samples), a detection kit 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.
- 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 Control Slides – Human Cell Pellet Cat. No. 310045, or Mouse 3T3 Cell Pellet Cat. No. 310023.
- RNAscope Introductory Pack designed for first time users of the RNAscope assay (Cat. No. 322435 for Human; Cat. No. 322436 for Mouse). The pack provides a complete set of reagents including controls probes, slides, the ImmEdge pen, and the RNAscope 2.5 Duplex Reagent Kit.
- VectaMount mounting media (Cat. No. 321584) is required for the RNAscope 2.5 HD Duplex Assay. Do not use any other mounting media.



### Reagents/Materials provided by Researcher

- Superfrost® Plus slides (Fisher Scientific) are required. Other slide types may result in tissue detachment.
- Gill's Hematoxylin I: dilute 1 : 1 with distilled water for counter staining.
- Fresh reagents (e.g. ethanol and xylene).
- Fresh 10% NBF (neutral-buffered formalin).
- Ammonium Hydroxide, effective concentration 0.02% (w/v).
- Hotplate, drying oven, water bath, thermometer, and microscope.
- Paraffin wax, microtome, and fume hood.
- 20x SSC if wish to stop after probe hybridization.

**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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