
MANUAL ASSAY: RNAscope® 2.5 Duplex Assay

CHK 47-004



Reagents/Materials required from ACD

- RNAscope Target Probes C1 and C2 Channel Probes
- RNAscope Duplex Positive Control Probe (PPIB-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 targeting the bacterial gene dapB 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 (which includes Hydrogen Peroxide, Target Retrieval and Protease Plus reagents), a detection kit, and wash buffer.
- HybEZ™ Hybridization System with ACD EZ-Batch Slide System Cat. Nos. 321461 and 321462 required for RNAscope hybridization to maintain optimum humidity and temperature at 40°C.
- RNAscope Introductory Pack designed for first-time users of the RNAscope assay (Cat. No. 322435 Human or Cat. No. 322436 Mouse), and providing a complete set of reagents including controls probes, slides, the ImmEdge® pen and the RNAscope 2.5 Duplex Reagent Kit.
- RNAscope Control Slides – Human Cell Pellet Cat. No. 310045, or Mouse 3T3 Cell Pellet Cat. No. 310023.
- ImmEdge Hydrophobic Barrier Pen (Cat. No. 310018) is the only pen that will maintain a hydrophobic barrier throughout the RNAscope procedure. No other pen should be used.
- VectaMount mounting media Cat. No. 321584
 - VectaMount Mounting media is a must-have reagent for the RNAscope 2.5 Duplex Assay. No other mounting media should be used.

**Reagents/Materials provided by Researcher**

- Superfrost® Plus slides (Fisher Scientific): Superfrost Plus slides are required. Other slide types may result in tissue detachment.
- Counter staining: diluting Gill's Hematoxylin 1:2 with distilled water is required for counter staining.
- 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, microscope, etc.
- Paraffin wax, microtome, 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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