

AUTOMATED ASSAY: RNAscope® Automated Assay on the Ventana™ Discovery™ Systems (ULTRA/XT) Doc. No. 322454-CKL-VS



Reagents/Materials required from Ventana/Roche Diagnostics

- mRNA Amplification, Pretreatment and Red/DAB Kits. These kits contain prefilled mRNA Detection reagents and the user fillable empty dispensers for all ACD reagents, except probes.

mRNA DAB Detection kit 06614353001

mRNA RED KIT: 7099037001

- Discovery wash buffer 950-510 to be used instead of EZ prep buffer
- Option 10 Dispenser 771-760 required for target retrieval buffer
- User-fillable probe dispensers. Cat. Nos. 05279828001, 05279836001, 05279844001 (960-761 through 960-780). You will need one for the negative control probe, one for the positive control probe and one for each of your target probes.
- RiboWash Buffer. This reagent (Cat. No. 760-105/05266262001) is required to run mRNA procedures on DISCOVERY instruments.
- mRNA DAB 4.0 staining procedure for DISCOVERY ULTRA or XT instrument
- Counterstain 1 and Counterstain 2 dispensers. Cat. Nos. 771-741 and 771-742.

Notes:

1. The following reagents are no longer required when running with RNAscope v 2.5 reagents: DISCOVERY 1x SSC, and DISCOVERY Inhibitor.
2. Unless specifically outlined above please continue to use the standard bulk reagents for the DISCOVERY ULTRA or XT instruments per your laboratory's standard workflow.
3. If you use your own hematoxylin and blueing reagents or the ones included in the ACD kit as complimentary, you will also need to order Counterstain 1 (771-741) and Counterstain 2 (771-742) user-fillable dispensers from Ventana.
4. If you have used the Ventana hematoxylin and blueing reagent for other applications, you can continue using those for the RNAscope VS assay.



Reagents/Materials required from ACD for RNAscope

CHECKLIST

- RNAscope 2.5 VS Reagent Kit – BROWN (Cat. No. 322200). Contains all the detection and pretreatment reagents needed to run the RNAscope assay on the Discovery Systems, except the RNA-specific probes.
- RNAscope 2.5 VS Reagent Kit – RED (Cat. No. 322250). Contains all the detection and pretreatment reagents needed to run the RNAscope assay on the Discovery Systems, except the RNA-specific probes.
- RNAscope 2.5 VS Positive Control Probe targeting common housekeeping gene to help qualify your samples and interpret your results (for Human – Hs UbC -3100049 or Hs PPIB 313909, or Hs Polr2a 310459; for Mouse – Mm Ubc 310779, Mm ppib 313919, or Mm polr2a 312479).
- RNAscope 2.5 VS Negative Control Probe targeting bacterial gene dapB gene to control for background noise and to help interpret assay results. Cat. No. 312049.
- RNAscope 2.5 VS Target Probes targeting specific RNAs of interest.
- RNAscope Control Slides – Human Cell Pellet Cat. No. 310045, or Mouse 3T3 Cell Pellet Cat. No. 310023.
- For offline boiling, use Cat No. 322000, RNAscope® Target Retrieval Reagents



Reagents/Materials provided by Researcher

- Superfrost® Plus slides (Fisher Scientific): Superfrost Plus slides are required. Other slide types may result in tissue detachment.
- Ecomount* or Cytoseal XYL mounting media:
 - Xylene based (such as CytoSeal XYL) Mounting media is required for the RNAscope VS Brown assay. Ecomount Mounting media is a must-have reagent for the RNAscope VS Red assay; no other mounting media can be used
- Fresh reagents (e.g. ethanol and xylene).
- Dawn detergent.
- Fresh 10% NBF (neutral-buffered formalin).
- Tissue-Tek® slide rack and staining dishes.
- 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>

CHECKLIST

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