RNAscope® VS Universal HRP and AP Automated Assay on the Ventana™ DISCOVERY™ ULTRA System

Doc. No. CHK 47-008

Note: This checklist includes consumables that are required for both the HRP-DAB assay and the AP-Red assay. If only one of these assays is run, only the required combination of Ventana and ACD reagent kits needs to be ordered.

Reagents/Materials required from Ventana/Roche Diagnostics

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

mRNA DAB Reagent Kits
- mRNA DAB Detection Kit (Cat. No. 760-224; Ordering Code 06614353001)
- mRNA Probe Amplification Kit (Cat. No. 760-222; Ordering Code 0661437001)
- mRNA Sample Prep Kit (Cat. No. 760-248; Ordering Code 08127166001)

mRNA Red Reagent Kits
- mRNA RED Detection Kit (Cat. No. 760-234; Ordering Code 7099037001)
- mRNA RED Probe Amplification Kit (Cat. No. 760-236; Ordering Code 07095341001)
- mRNA Sample Prep Kit (Cat. No. 760-248; Ordering Code 08127166001)

☐ DISCOVERY™ Wash Buffer (Cat. No. 950-510; Ordering Code 07311079001). Use instead of EZ Prep Buffer.

☐ User-fillable probe dispensers for negative control probe, positive control probe, and the target probe. Additional dispensers can be ordered for m target probes. See the Ventana™ website for part numbers.
- Cat. No. 960-761; Ordering Code 05279828001
- Cat. No. 960-762; Ordering Code 05279836001
- Cat. No. 960-763; Ordering Code 05279844001

☐ 10X SSC Solution (Cat. No. 950-110; Ordering Code 05353947001). Required to run mRNA procedures on the DISCOVERY™ ULTRA.

☐ Ultra LCS (Predilute) Cat. No. 650-210; Ordering Code 05424534001.
☐ Reaction Buffer (10X) Cat. No. 950-300; Ordering Code 05353955001.

☐ Discovery CC1 (Cat. No. 950-500; Ordering Code 06414575001).

☐ mRNA Universal staining procedure for DISCOVERY™ ULTRA (which can be installed by Roche or ACD FAS)

☐ Counterstaining reagents.
  • Hematoxylin II (Cat. No. 760-2208; Ordering Code 05277965001)
  • Bluing (Cat. No. 760-2037; Ordering Code 05266769001)

**Note:** If ACD’s or one’s own hematoxylin and bluing reagents are used, Counterstain 1 (Cat. No. 771-741) and Counterstain 2 (Cat. No. 771-742) user-fillable dispensers from Ventana™ will need to be ordered.

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**Reagents/Materials required from ACD for RNAscope**

☐ RNAscope® VS Universal HRP Reagent Kit – BROWN (Cat. No. 323200). Contains the following:
  • RNAscope® VS Universal HRP Detection Reagents (Cat. No. 323210)
  • RNAscope® VS Accessory Kit (Cat. No. 320630)
  • RNAscope® VS Universal Sample Prep Reagents v2 (Cat. No. 323740)

☐ RNAscope® VS Universal Reagent Kit – RED (Cat. No. 323250). Contains the following:
  • RNAscope® VS Universal AP Detection Reagents (Cat. No. 323260)
  • RNAscope® VS Accessory Kit (Cat. No. 320630)
  • RNAscope® VS Universal Sample Prep Reagents v2 (Cat. No. 323740)

☐ RNAscope® 2.5 VS Positive Control Probe (choose species needed): Cat No. 312029 for Human **UBC**; Cat No. 313909 for Human **PPIB**; Cat No. 310459 for Human **POLR2A**; Cat No. 310779 for Mouse **Ubc**; Cat No. 313919 for Mouse **Ppib**; Cat No. 312479 for Mouse **Polr2a**.

☐ RNAscope® 2.5 VS Negative Control Probe (Cat. No. 312039). Targets the bacterial **dapB** gene to control for background noise, and to help interpret assay results.

☐ RNAscope® 2.5 VS Target Probes targeting specific RNAs of interest.

☐ RNAscope® Control Slides. Choose species needed (Cat. No. 310045 for Human Cell Pellet; Cat. No. 310023 for Mouse 3T3 Cell Pellet).

☐ Use RNAscope® Target Retrieval Reagents to boil samples offline (Cat No. 322000). This is included in the Reagent Kits.
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:
  - Use EcoMount mounting media with the RNAscope® VS Universal AP Assay; no other mounting media can be used
  - Use a xylene-based mounting media (such as CytoSeal XYL) with the RNAscope® VS Universal HRP Assay.

- 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, and microscope

- Paraffin wax, microtome, 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 https://acdbio.com/technical-support/user-manuals.