
RNAscope® 2.5 Automated Assay on Leica Biosystems' BOND RX

Doc. No. CHK 47-016

Note: This checklist includes consumables that are required for both the BROWN-DAB assay and the RED-AP assay. If only one assay is ran, only the required combination of Leica and ACD reagent kits need to be ordered.



Reagents/Materials required from Leica Biosystems

- BOND Polymer Refine Detection (DAB or Red) and Hematoxylin. These kits contain prefilled chromogens DAB (Cat. No. DS9800) or Red (Cat. No. DS9390), and Hematoxylin reagents.
- BOND Open Containers (Cat. No. Op309700). Fill these containers with reagents from the RNAscope LS Reagent Kit.
- BOND Universal Covertiles (Cat. No. S21.2001).
- BOND Dewax Solution (Cat. No. AR9222). Place solution in one of the large containers located in the bottom of the instrument.
- BOND Epitope Retrieval Solution 1 and 2 (Cat. Nos. AR9961 and AR9640).
- BOND Wash Solution 10X (Cat. No. AR9590). Dilute solution 1:10 and place in one of the large containers located in the bottom of the instrument.
- BOND Aspirating Probe Cleaning System (Cat. No. CS9100).
- BOND Mixing Stations (Cat. No. S21.1971). Replace every month.
- BDZ 9.0 or above versions.



Reagents/Materials required from ACD for RNAscope

- RNAscope® 2.5 LS Reagent Kit – BROWN (Cat. No. 322100) or RNAscope 2.5 LS Reagent Kit – RED (Cat. No. 322150). Contains all the detection and pretreatment reagents needed to run the RNAscope assay on the Leica BOND RX, except the RNA-specific probes.
- RNAscope® 2.5 LS Positive Control Probe targeting common housekeeping gene to help qualify the samples and interpret the results (for Human – Hs UBC -312028, Hs PPIB-313908 or Hs POLR2A-310458; for Mouse – Mm Ubc-310778, Mm Ppib-313918, or Mm Polr2a-312478).

- RNAscope® 2.5 LS Negative Control Probe targeting bacterial gene *dapB* gene to control for background noise and to help interpret assay results (Cat. No. 312038).
- RNAscope® 2.5 LS Target Probes targeting specific RNAs of interest.
- RNAscope® Control Slides – Human Cell Pellet (Cat. No. 310045) or Mouse 3T3 Cell Pellet (Cat. No. 310023).
- Ecomount (for LS Red Assay. Ecomount Mounting media is required; do not use any other mounting media
- Software protocols created for each probe (workaround). Please ask FAS for the most appropriate protocols for the RNAscope® Assay.



Reagents/Materials provided by Researcher

- Superfrost™ Plus slides (Fisher Scientific): Superfrost Plus slides are required. Other slide types may result in tissue detachment.
- Cytoseal XYL mounting media:
 - Use a xylene-based mounting media (such as CytoSeal XYL) with the RNAscope® 2.5 LS-Brown Assay.
- Fresh reagents (e.g. ethanol and xylene)
- 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>.