
Checklist for the RNAscope® Automated Assay on Leica Biosystems' BOND RX System RNAscope® 2.5 LS Duplex Assay

Doc. No. CHK 47-001



Reagents/Materials required from Leica Biosystems

- BOND Polymer Refine Detection Kits (DAB kit, Cat. No. DS9800; Red kit, Cat. No. DS9390). These kits contain prefilled DAB, Red, and Hematoxylin reagents.
- BOND Open Containers (Cat. No. Op309700). Fill these containers with reagents from the RNAscope LS Duplex 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 with DI or MilliQ water and place in one of the large containers located in the bottom of the instrument.
- BOND Mixing Stations (Cat. No. S21.1971). Replace every month.
- BOND DAB Enhancer 30ml (Cat. No. AR9432).
- BOND Aspirating Probe Cleaning System (Cat. No. CS9100).
- BDZ 11.0 software.



Reagents/Materials required from ACD for RNAscope®

- RNAscope® 2.5 LS Duplex Reagent Kit (Cat. No. 322440). The kit contains all the detection and pretreatment reagents needed to run the RNAscope assay on the Leica BOND RX, except for the RNA-specific probes.
- RNAscope® 2.5 LS Duplex Positive Control Probe (Cat. No. 320748 for Human PPIB-C1/Polr2A-C2; Cat No. 320768 for Mouse PPIB-C1/POLR2A-C2). Probes target common housekeeping genes to help qualify your samples and interpret your results.
- RNAscope® 2.5 LS Duplex Negative Control Probe (Cat. No. 312758). The probe targets the bacterial *dapB* gene in two channels to control for background noise, and to help interpret assay results (*dapB* C1/*dapB*-C2).

- RNAscope[®] 2.5 LS Target Probes target specific RNAs of interest in Channel C1 and Channel C2.
- RNAscope[®] Control Slides (Cat. No. 310045 for Human Cell Pellet; Cat. No. 310023 for Mouse 3T3 Cell Pellet).
- Software protocols created for each probe (workaround solution).



Reagents/Materials provided by Researcher

- Superfrost[®] Plus slides (Fisher Scientific): Superfrost Plus slides are required. Other slide types may result in tissue detachment.
- Fresh reagents (e.g. ethanol and xylene).
- Fresh 10% NBF (neutral-buffered formalin).
- Tissue-Tek[®] slide rack and staining dishes.
- EcoMount or PERTEX[®].
- 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 <http://www.acdbio.com/technical-support/user-manuals>.