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## Checklist for Manual RNAscope<sup>®</sup> HiPlex Assay

Doc. No. CHK 47-017

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### Reagents/Materials required from ACD

- RNAscope<sup>®</sup> HiPlex Target Probes with Tail designation T1 to T12.
- RNAscope<sup>®</sup> HiPlex Probe Diluent (Cat. No. 324301).
- Species-specific RNAscope<sup>®</sup> HiPlex 12 Positive Control Probes. Probes target 12 common housekeeping genes to help qualify samples and interpret results (Mouse, Cat No. 324321; Rat, Cat No. 324331, etc)
- RNAscope<sup>®</sup> HiPlex 12 Negative Control Probe (Cat. No. 324341). The probe targets the bacterial *dapB* gene to control for background noise and help interpret assay results.

Order ONE depending on the wavelengths the imaging system can support:

- RNAscope HiPlex12 Reagents Kit (488, 550, 647) (Cat. No. 324108). Each kit contains: RNAscope<sup>®</sup> HiPlex12 Detection Kit (488, 550, 647) (PN 324106), RNAscope<sup>®</sup> Target Retrieval Reagents (PN322000) RNAscope Protease III & Protease IV (PN 322340), RNAscope Wash Buffer (PN 310091), and HiPlex Cleaving Stock Solution (PN 324130).

OR

- RNAscope HiPlex12 Reagents Kit (488, 550, 647, 750) (Cat. No. 324107) Each kit contains: RNAscope<sup>®</sup> HiPlex12 Detection Kit (488, 550, 647, 750) (PN 324105), RNAscope<sup>®</sup> Target Retrieval Reagents (PN322000) RNAscope Protease III & Protease IV (PN 322340), RNAscope Wash Buffer (PN 310091), and HiPlex Cleaving Stock Solution (PN 324130).
- HybEZ<sup>™</sup> Hybridization System with ACD EZ-Batch Slide System II (Cat. Nos. 321720 or 321710). The system maintains optimum humidity and temperature at 40°C during RNAscope<sup>®</sup> hybridization.
- ImmEdge<sup>™</sup> Hydrophobic Barrier Pen (Cat. No. 310018) is the only pen that will maintain a hydrophobic barrier throughout the RNAscope procedure. Do not use any other pen.
- RNAscope<sup>®</sup> HiPlex Image Registration Software for registering the images acquired during iterative detection.



## Reagents/Materials provided by Researcher

- Superfrost<sup>™</sup> Plus slides (Fisher Scientific cat# 12-550-15) are required. Other slide types may result in tissue detachment.
- Coverslips (Fisher Scientific cat# 12-545F).
- ProLong<sup>®</sup> Gold Antifade Reagent from ThermoFisher (P36930).
- Fresh reagents:
  - 100% EtOH
  - 10X PBS
  - 10% Tween 20
  - 20X SSC
  - DI/RO water
- Fresh 10% NBF (neutral-buffered formalin) or 4% PFA (paraformaldehyde).
- Water bath, thermometer, and epi-fluorescent or confocal microscope capable of imaging DAPI, AF 488, ATTO 550, ATTO 647N, and AF 750. For Cat. No. 324108, AF 750 imaging is not required.
- Cryostat and fume hood.
- Steamer or hot plate/beaker and digital thermometer (for samples requiring target retrieval).

**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>.