

IN SITU HYBRIDIZATION & RNAscope SERVICES

Mouse Histology & Phenotyping Laboratory

The MHPL provides In Situ Hybridization (ISH) services for the detection of nucleic acid targets. Our most popular service is RNAscope for the detection of RNA targets. MHPL recommends FFPE (formalin-fixed paraffin-embedded) tissues for better preservation of RNA integrity, morphology, convenient storage of specimens, and robustness of tissue sections for the lengthy ISH procedure.

POINTERS FOR PREPARING FFPE SPECIMENS

1. If possible, use intracardiac systemic perfusion fixation with 10% neutral buffered formalin (NBF) followed by immersion fixation of dissected tissues. This method has 2 advantages:
 - a. It flushes out blood from organs and tissues which is a major source of autofluorescence
 - b. The method promptly and thoroughly fixes organs and is particularly important for CNS and cell-dense tissues.
2. Tissue specimens should be fixed in fresh 10% neutral buffered formalin (NBF) (10-20x volume of the tissue) for 16-36 hours at room temperature and blocked into a thickness of 3-4 mm.
3. Rinse organs thrice for 5 min each with RNase-free 1x PBS.
4. Store organs at 4°C in 70% ethanol prepared using RNase-free H₂O.
5. Submit for paraffin tissue processing promptly to avoid RNA degradation.
6. FFPE blocks should be sectioned under RNase-free conditions.
 - a. RNase-free sectioning service is available at MHPL
 - b. TOMO slides are recommended for robust adhesion of FFPE sections.

REAGENTS THAT MUST BE SUBMITTED BY MHPL USERS

Users requesting ISH/RNAscope and related services are expected to purchase the following reagents directly from the vendor and submit them to MHPL with their order:

1. Species-specific experimental probes. Please check the following link for available probes:
https://www.bio-techne.com/search?search_type=products&keywords=RNAscope%20probes
Please contact Kari Bonner (kari.bonner@bio-techne.com) if custom probes are needed.
2. Appropriate Detection Kit (please choose accordingly). Each of the following kits contains enough reagents for 20 slides with tissue sections (20 mm x 20 mm area):
 - a. RNAscope Multiplex Fluorescent Detection Kit v2 for up to 3 mRNA targets (ACD Cat# 323110)
 - b. RNAscope Plus smRNA-RNA HD Reagents Kit, Fluorescent Detection Kit for co-detection of smRNA (miRNA or siRNA) and up to 3 different mRNA targets (ACD Cat# 322785)

Please contact Kari Bonner (kari.bonner@bio-techne.com) to obtain quotes for probes and detection kits.

REAGENTS INCLUDED WITH ISH/RNAscope SERVICE BY MHPL

The following reagents are provided by MHPL and need not be ordered by requesters:

1. RNAscope Wash Buffer
2. RNAscope Antigen Retrieval Buffer
3. TSA Fluorophores and TSA buffer
4. Positive and Negative Control Probes (for mouse and human tissues only)