HOPE Tissue Blocks:

OVERVIEW

HOPE is a new tissue preservative method that maintains RNA integrity and still allows blocking of tissue into paraffin. The paraffin-blocked tissue can then be cut into sections on a standard microtome. This method of tissue preservation does not completely denature or cross-link structural proteins, enzymes or nucleic acids, thus maintaining RNA, protein and DNA integrity. However, this fixation method does not inactivate viruses, prions and microorganisms, etc. You should therefore treat HOPE-fixed tissue as you would fresh tissue. All HOPE tissue should be considered potentially infectious, so follow standard blood-borne pathogen precautions when dealing with HOPE specimens. Below we have provided some specialized handling information that should help you to optimize your assay results when using Hope-fixed tissue:

Storage:

*HOPE blocks must be stored in a refrigerator (4 degrees C) until sectioning is performed.

Deparaffinization:

*You can use a standard deparaffinization protocol of xylene, graduated alcohols and deionized water for typical hematoxylin and eosin staining. An alternative method is to use isopropanol (60ºC) for ten minutes, washing thoroughly with fresh, warm isopropanol.

*When planning to use Hope-fixed tissue to identify enzymes, DNA or RNA in the tissue, use hexamethyldisiloxan for deparaffinization as this method is especially gentle.

Rehydration:

*Incubate slides for ten minutes in the refrigerator in 70% cool acetone. Wash slides twice in deionized water.

H&E Staining:

*Transfer rehydrated sections to hematoxylin for 2-4 minutes, washing in deionized water thoroughly. Bluing reaction can be performed under running tap water (1-2 minutes) followed up by washing with deionized water.

*Avoid any influence of acids, e.g. HCI/alcohol. Incubate in eosin for 30 seconds to a few minutes – depending on intensity desired. Wash twice in deionized water, dehydrating quickly dipping the slides in 70% Isopropanol x2, absolute abs x2 and incubate in absolute alcohol for ten minutes. Briefly wash with xylene x2, drip dry and coverslip.

Immunohistochemistry:

*Antigen retrieval is not necessary for HOPE specimens.

*Never block endogenous peroxidase in a methanol solution with peroxide when using HOPE specimens. This can potentially destroy the antigen.

*Primary antibody and detection systems can, in many cases, be used at lower concentrations with HOPE specimens.

*Use an aqueous medium when mounting HOPE specimens.

In situ-Hybridization (ISH):

*If at all possible, avoid SDS and dextran sulfate in preparing the hybridization mix. Enzymatic digestion is hardly ever necessary.

*As pre-treatment for ISH, incubate slides in a pre-warmed, probe-specific buffer (per the manufacturer recommendations), in a 95º warm water bath for 5-7 minutes. The time counts only after reaching final temperature.

*Posthybridization: Incubate slides twice for 2 minutes in 70% 2-8º cold acetone, let them air dry and cover-slip as usual.

Laser Microdissection:

*Sterilization of disposable blades and tweezers should be done by baking at 200ºC. Use sterile Eppendorf® cups. For every single tissue block, use a new blade section. Execute all steps with gloves and use all DEPC-treated water solutions.

*For higher yields of nucleic acids, sections of a thickness of 15-20 µm can be used. Drying at 37-56º is recommended.

RNA/DNA Analysis

Slide cutting: the following procedures should be done following standard RNA free isolation protocols as much as possible.

Note: to preserve RNA integrity in cut sections of HOPE-fixed tissue we currently recommend storing slides at -20˚C and avoiding water condensation.

Deparaffinization protocol:

1. Cut sections 5–10 µm thick. (Discard the first 2–3 sections)
2. Dry the sections in an incubator at 50˚C for 30 min or preferably at 37˚C overnight.
(If you plan to proceed immediately to isolation, shake off the excess water and avoid the drying step)
3. Use a single edge blade to trim excess paraffin off the sample block. Run samples through xylene and ethanol.

Options for deparaffinization

Option 1:

1a) Incubate in xylene for 30sec. Shake off the xylene after incubation. Incubate for 30sec with 100% ethanol and shake off excess after incubation. *(The ethanol extracts residual xylene from the sample) (Be careful not to drop the tissue from the slide during shaking).*
1b) Incubate for 5 min with xylene. Shake off excess xylene from the slide and dry sample under the hood for 5 min.

Option 2:

2a) Immediately place the sections in a 1.5 or 2 ml microcentrifuge tube and close the lid.
2b) Add 500µl xylene to the sample. Vortex vigorously for 10 seconds. Incubate for 20 seconds.
2c) Centrifuge at full speed (>16,000g) for 2 min at 20–25ºC.
2d) Remove the supernatant by pipetting. Do not remove the pellet.
2e) Add 500µl of 100% ethanol to the pellet, mix by vortexing and incubate for 20 seconds.
2f) Centrifuge at full speed for 2 min (>16,000g) at 20–25ºC.
2g) Remove the supernatant by pipetting. Do not remove the pellet. Carefully remove any residual ethanol and dry sample under the hood for 5 min.

Option 3:

3a) Incubate sections for 10 minutes in xylene in a microcentrifuge tube. Empty the xylene and add new xylene and reincubate for 10 minutes. Centrifuge for 10 minutes (21000) g at 20–25ºC.
• Repeat the above steps with ethanol (two incubations followed by centrifugation).

• Hexamethyldisiloxan can be used for deparaffinization and may enhance protection of the nucleic acids.

Isolations of RNA/DNA/Protein can proceed accordingly to protocols designed for specific isolation.

Note regarding RNA isolation: If using Qiagen MinElute kits keep columns at 4˚C until needed and proceed to do the experiment at room temperature once you have reached the column isolation steps.