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.

Formats

The bank has three different formats for HOPE tissue. You may request a paraffin embedded block, slices or slides. A **Block** is similar to a formalin-fixed-paraffin embedded block and may be cut on a microtome. **Slides** are approx 5 micron thick sections of HOPE fixed, paraffin embedded tissue placed on a charged glass slide suitable for immunohistochemistry or other procedures that require a thin slice of tissue attached to a glass slide. **Slices** are 20-micron thick sections of paraffin-embedded tissue that are curled into a microfuge tube. These sections are suitable for isolating RNA or DNA or protein or any procedure that does NOT require being attached to a glass slide. **Slices** can NOT be put on a slide and can NOT be used for staining or laser micro dissection. Please contact the Tissue Core Lab if you have any questions about the proper format for your particular experiment.

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 they are sectioned.

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.

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 5 minutes then wash thoroughly in deionized water. Bluing reaction can be performed by running under tap water (4 minutes). Avoid using any acids, e.g. HCl/alcohol.

* Incubate in eosin for 30 seconds to a few minutes – depending on intensity desired. Dehydrate by quickly dipping the slides in absolute alcohol 15 times. Briefly wash with xylene x2, drip dry and coverslip.

Immunohistochemistry:

*Antigen retrieval is rarely necessary for HOPE specimens.

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

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 of slide 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 slide. This decreases the amount of paraffin that needs to be removed from the sample to avoid interference in the isolation steps. Run samples through xylene and ethanol following one of the options listed below.

Options for deparaffinization

Option 1: Sections remain on slide

1a) Incubate slide 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.

OR: 1b) Incubate for 5 min in xylene. Shake off excess xylene from the slide and dry sample under the hood for 5 min.

Option 2: Tissue Removed from Slide

2a) Immediately scrape the section off of the slide with a razor blade and place the sections in a 1.5 or 2 ml microcentrifuge tube.

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) Pipet off the supernatant. Do not remove the pellet.

2e) Add 500µl of 100% ethanol to the pellet, vortex and incubate for 20 seconds.

2f) Centrifuge at full speed for 2 min (>16,000g) at 20–25ºC.

2g) Pipet off the supernatant. Do not remove the pellet. Carefully remove any residual ethanol and dry sample under the hood for 5 min.

Option 3: Tissue Removed from Slide

3a) Scrape tissue off of the slide and incubate 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).

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

HOPE solution may be purchased from **DCS** Innovative Diagnostik-Systeme (http://www.dcs-diagnostics.de) in Germany, or their American distributor:

Polysciences, Inc.
400 Valley Road
Warrington, PA 18976
phone: (800)523-2575 or (215)343-6484
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Both websites have further protocols and FAQ’s available on their websites.