コスモ・バイオ株式会社

Polysciences, Inc.
Chemistry beyond the ordinary

Corporate Headquarters
400 Valley Road
Warrington, PA 18976
1-800-523-2575
FAX 1-800-343-3291
Email: info@polysciences.com
www.polysciences.com

Europe - Germany
Polysciences Europe GmbH
Handelsstr. 3
D-69214 Eppelheim, Germany
(49) 6221-765767
FAX (49) 6221-764620
Email: info@polysciences.de

TECHNICAL DATA SHEET 767

HOPE® Fixative

Hepes Glutamic Acid Buffer Mediated Organic Solvent Protection Effect

System I & System II

Cat. #24823 & #24824

The HOPE® technique is mainly used for the preparation of paraffin embedded tissue sections. In contrast to other fixation methods HOPE® does not completely denature or cross-link structural proteins, enzymes, and nucleic acids. They remain in an almost native state. This means that HOPE®- fixed tissue can also include active viruses, prions, microorganisms etc. **HOPE®- fixed tissue blocks, paraffin sections etc. must therefore be considered as potentially infectious** unless suitable tests have proven otherwise. **Always wear protective-gloves!**

HOPE® reagents contain up to 0.03 % NaN3 (sodium azide). Sodium azide is not classified as hazardous at the concentration used in these products. However, toxicity of sodium azide at the products' concentration has not been thoroughly investigated.

1. Tissue Fixation and Preparation of Paraffin Blocks

- Transfer tissue after resection immediately into sterile plastic petri dish and keep cool. Take care that the tissue does not dry out. Seal the dish with Parafilm if necessary. Cut tissue into pieces of max. 8mm x 8mm x 8mm and transfer into disposable 5ml tubes with ice-cold (0-4°C) HOPE® I.

  Important: To ensure good diffusion and penetration of the tissue, only use cut/sectioned organs and organ parts, respectively, i.e. do **NOT** use encapsulated tissues! If using frozen tissue, do NOT thaw prior to transfer into HOPE® I solution. Using frozen tissue as starting material can lead to a lower quality of morphology compared to fresh tissue.

- **Incubate tissue in HOPE® I solution at 0-4°C for 12-72 hours.** During this incubation, erythrocytes might diffuse out of the tissue and form a reddish pellet in the tube. However, this does not affect the quality of the fixation. The color of HOPE® I should stay orange-yellow throughout the fixation representing a stable pH.
  For processing tissue samples with high cell density (like brain or lymph nodes) incubation in HOPE® I solution for at least 40 hours is recommended.

- After incubation remove HOPE® I. **Add 5ml pre-mixed ice-cold HOPE® II / acetone solution (100 ml acetone + 100 µL HOPE® II) to tissue and transfer immediately back into refrigerator or ice bath.** Acetone resistant tubes should be used from this step on. Occurence of a white precipitate does not affect quality of fixation. Incubate at 0-2°C (i.e. on ice) for 2 hours.
  NOTE: The quality of the fixation and the performance during cutting of the paraffin-embedded HOPE®-fixed tissue is critically dependent on the acetone temperature in this step. Avoid acetone temperatures above 2°C.

- After 2 hours, discard HOPE® II / acetone solution and **re-fill this time with ice-cold pure acetone. Incubate for 2 hours. Repeat this pure acetone step twice** with each incubation lasting 2 hours.

- After 8 hours of dehydration overall, discard acetone and immediately **add pre-warmed low-melting paraffin**. (Peel-A-Way® MicroCut Paraffin 52°- 54°C, Cat. #24201) Make sure tissue does not dry out in between steps. Avoid trapping air bubbles in the paraffin. **Incubate overnight at exactly 54-55°C**.

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If at this point the paraffin has not completely penetrated the tissue, the process cannot be optimized anymore by longer incubation. Re-embed tissue as usual using fresh paraffin. In doing so avoid any air bubbles. When processing small tissue samples and paraffin volumes it is recommended to store the paraffin vials in a preheated thermoblock immediately after taking them out of the 54°C incubator to avoid early solidifying of the paraffin.

Table: Overview fixation steps

Reagent	Incubation time
HOPE® I	12-72 hours
HOPE® II / acetone	2 hours
acetone	2 hours
acetone	2 hours
acetone	2 hours

- Store blocks **in refrigerator** until sectioning.

- Advice for transportation of tissue samples for HOPE®-fixation: Technical protocols for transport of tissue material for HOPE®- fixation are still under development. We recommend to transfer the fresh tissue immediately into cold HOPE® I and incubate for at least 16 hours at 4°C. If, during the subsequent transport, the tissue stored in HOPE® I becomes slightly warmer, no negative impact on morphology has been observed up to now.

2. Sectioning of HOPE®-Fixed Paraffin-Embedded Tissue

Please, take the time to practice the cutting technique with HOPE®-fixed tissue with less important material!

- Store paraffin blocks at about -20°C for 30 minutes and fix on microtome as usual. Prepare two different water baths and set #1 at RT, #2 at 35-39°C or use a heating-plate alternatively.

- Collect sections in the water bath #1 (RT). Lift out sections with clean or coated slide and, **one by one**, stretch them out on the surface of water bath #2 (35-39°C) or heatplate. NOTE: Sections tend to spread easily because there is no cross-linking between protein structures. As soon as section is stretched and wrinkle-free on slide, take it out of the water bath, store them in a slide rack. Dry sections in an incubator at 50°C for about 30 minutes or (preferably at 37°C overnight). Dried sections can be stored in the refrigerator without problems for a long peroid of time. Blocks should be stored at 4°C.

3. Dewaxing, Rehydration and H&E Staining

- For deparaffinization, place slides into first cuvette with isopropanol (approx. 60°C). Incubate for 10 minutes. Wash thoroughly with fresh, warm isopropanol. For rehydratization incubate slides twice for 10 minutes in the refrigerator in 70% cool (2-8°C) acetone. Wash slides twice with deionized water, then store for 5 minutes in a cuvette with deionized water.

- Alternatively use hexamethyldisiloxan for deparaffinization. This method is specially gentle for enzymes, and the analysis of DNA and RNA.

- **To stain with H&E**, transfer rehydrated sections to hematoxylin *(Harris Hematoxylin, Cat. #24245, Mayers Hematoxylin, Cat. #24821)* for 2-4 minutes. Wash thoroughly in a cuvette with deionized water and transfer to a second cuvette with deionized water. Perform bluing reaction under running tap water (1-2 minutes) or in ammoniac water 1:500 with following washing with deionized water. **Important: Avoid any influence of acids (i.e. HCl/alcohol)!** Incubate in eosin for 2-4 minutes depending on the intensity desired. Wash twice in deionized water and dehydrate rapidly dipping the slide into the following solutions: 2x 70% **Isopropanol**, 2x **alcohol abs**. Incubate for another 10 minutes in a third cuvette with alcohol absolute. Briefly wash in cuvette with xylene, then transfer into a second cuvette of xylene and incubate for 5 minutes. Take out, drip-dry and coverslip.

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- **Note: If the HOPE®-fixed tissue sections are prepared for H&E staining only, dewaxing and rehydration can be done as routinely in xylene/ethanol. Subsequent H&E staining can be performed as usual, for example in automated stainers. Slides dewaxed and rehydrated in xylene/ethanol are suitable for other downstream applications like immunohistochemistry (IHC) or *in situ*-hybridization (ISH), as well. But please be aware that some major advantages of the HOPE®-fixation will be neutralized by this procedure. For example after xylene/ethanol treatment even HOPE®-fixed tissue often requires HIER (Heat-induced epitope retrieval) for IHC staining and primary antibodies and detection reagents have to be applied in higher concentrations.**

4. Immunohistochemistry (IHC) on HOPE®-Fixed Tissue Sections

In principle conventional streptavidin/biotin systems can be used for immunohistochemistry. Sometimes they result in a higher background staining. If the level of background is inacceptable suitable blocking steps or biotin-free detection systems should be preferred.

Comment: Peroxidase-based detection systems are recommended for HOPE®- fixed mouse tissue, since endogenous alkaline phosphatase activity in mouse tissues can not be blocked completely even when using levamisole.

- To **block endogenous peroxidase**, incubate deparaffinized and rehydrated tissue sections (preparation see sections 2 and 3) for 10 minutes in a cuvette with 0.5 % H_2O_2 (in deionized water). **NEVER block endogenous peroxidase in MetOH solution with peroxide (H_2O_2), since this is highly likely to destroy antigen structures (epitopes)**. Wash slides thoroughly with PBS. (Note: Never allow sections to dry out during the whole staining procedure!)

- Stain slides as usual with primary antibody and detection system / chromogen without pretreatment. Primary antibody and detection system components can in many cases be used at lower concentrations (sometimes one-third of the normal concentration) as used for formalin fixed tissues.

- Optionally heat the slides in pre-warmed citrate buffer in a water bath for 5 minutes. The concentration of antibody and detection system could be reduced further.

- After immunhistochemical staining counterstain the slides as usual and mount with aqueous medium. Counterstain of nuclei seems to be less stable in glycerol gelatine, therefore synthetic mounting media should be preferred.

5. *In situ*-Hybridization (ISH) on HOPE®-Fixed Tissue Sections

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

- Cut the slides as described above and warm-up on a 70°C heating-plate (i.e. Omnislide) for 10 minutes before dewaxing. Deparaffination and rehydratization is performed as described above.

- As **pretreatment for *in situ*-hybridization** incubate slides in pre-warmed, probe specific buffer (as recommended by the manufacturer), in a 95°C warmed water bath for 5-7 minutes. The time counts only after reaching final temperature. After heating step, let the cuvette cool down for 15 minutes. Wash slides for 2-3 minutes in buffer. Let the slides air dry and place them then for 2 minutes in 2 - 8°C cold 70% acetone. Let the slides air dry again.

- Hybridization with probe and signal amplification (if necessary) are to be carried out according to established protocols.

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

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6. Analysis for DNA/RNA

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

- Cut from the block 1 to 4 sections of 5µm thickness for each sample:

    - Incubate sections 2x for 10 minutes at room temperature in xylene (e.g. in a Eppendorf® cup) and then centrifuge (10 minutes at 21000 g at room temperature).

    - Incubate sections 2x for 10 minutes at room temperature in ethanol (e.g. in a Eppendorf® cup) and then centrifuge (10 minutes at 21000 g at room temperature)

- Alternatively, hexamethyldisiloxan can be used for deparaffination for enhanced protection of the nucleic acids.

- Solubilize the tissue sections after the last centrifugation in appropiate buffer depending on used extraction method (e.g. RNEasy or Trizol). Use of DNA / RNA purification-columns or magnetic beads is possible.

7. Laser Microdissection

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

- Cut sections of 4-10µm thickness. For higher yields of nucleic acids, sections of a thickness of 15-20µm can be used. Mount sections and air dry. Drying at 37 to 56°C is recommended.

- Deparaffinate slides as described above. For preparations, which are not to be stained, deparaffination is recommended too, because paraffin reduces the efficacy of the laser cutting. The following staining with H&E is to be carried out as described above. After the steps with isopropanol let the slides air dry and store them cool. For best results mount, deparaffinate and stain sections shortly prior laser sectioning.

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Ordering Information

Cat. #	Description	Size
24823-500	HOPE® Fixative - System I	500ml
24823-2500	HOPE® Fixative - System I	2500ml
24824-1	HOPE® Fixative - System II	1ml

Additional Products:

08523	Acetone, Glass Distilled, 99.7%	1L / 4L
01921	Acetone, EM grade, 99.5%	6pints / 1L / 4L
09860	Alcohol Reagent (100%), histology grade	1 Gal
24079	0.05M Phosphate-Citrate Buffer, pH 5.0±0.2, 1X Powdered Blend	5pk / 10pk
19562	Peel-A-Way® MicroCut Paraffin 52°-54°	(4 x 2.5kg) case
3989A	Parafilm, 4" x 250'	1 roll
3989C	Parafilm, 2" x 250'	1 roll
24821	Mayers Hematoxylin	
24245	Harris Hematoxylin, Acidified (mercury-free)	500ml / 1000ml
17269	Eosin Y, 1% alcoholic solution	500ml / 1000ml
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