Electron
Microscopy
Sciences

INSTRUCTIONAL MANUAL
CAT. #26750 Series
EMS Rapid Pro Processing System

Protocol:
Paraffin Manual Processing for Fresh and Pre-treated Tissue

Electron Microscopy Sciences
P.O. Box 550 ♦1560 Industry Road ♦ Hatfield PA 19440
www.emsdiasum.com
Toll Free: **1-800-523-5874**
sgkcck@aol.com
Tel: 215-412-8400 ♦ *Fax:* 215-412-8450

Protocol for Paraffin Manual Processing for Fresh and Pre-treated Tissue

Special Notes

- The tissue can be, but does not necessarily have to be, pretreated for about 2 hours with routine or EMS Rapid Pro Processing formalin. Usually when it is collected in medical office settings and has to be delivered to a laboratory.
- The fresh tissue can be placed in EMS Rapid Pro Processing System (ERPPS) solutions and processed immediately, usually in a hospital setting or any facility where pathology lab is available.

Microscopic analysis of cells and tissues requires the preparation of very thin, high quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures. Most fresh tissue is very delicate and easily distorted and/or damaged and it is thus impossible to prepare thin sections from it unless it is chemically preserved or “fixed” and supported in some way whilst it is being cut.

Tissue processing can be performed manually (hand processing), but where multiple specimens have to be dealt with it is more convenient and much more efficient to use an automated tissue processing machine (a “tissue processor”). Most modern fluid-transfer processors employ raised temperatures, effective fluid circulation and incorporate vacuum/pressure cycles to enhance processing and reduce processing times.

Overview of Tissue Processing for Paraffin Sections

Obtaining a Fresh Specimen

Fresh tissue specimens will come from various sources. It should be noted that they can very easily be damaged during removal from subject. It is important that they are handled carefully and appropriately fixed as soon as possible after dissection. Ideally fixation should take place at the site of removal, perhaps in the operating theatre, or, if this is not possible, immediately following transport to the laboratory.

Fixation

The specimen is placed in a liquid fixing agent (fixative) such as ERPPS's solution. This will rapid penetrate the tissue causing chemical and physical changes that will harden and preserve the tissue and protect it against subsequent processing steps. Fixative volume should be 20 times that of tissue on a weight per volume. Due to the fast rate of diffusion of solution (1mm per 30-60 seconds) tissue should be sectioned into 2mm for biopsy, and 4mm round punch slices. Generally this will mean that the specimen should fix for 3 min. Most laboratories will use a fixative step as the first station on their processor.

Specimens that are to be processed will be placed in suitable labelled cassettes (small perforated baskets) to segregate them from other specimens. The duration of the processing schedule used to process the specimens will depend on the type and dimensions of the largest and smallest specimens, the particular processor employed, the solvents chosen.

Dehydration, Clearing and Infiltration

Because melted paraffin wax is hydrophobic (immiscible with water), most of the water in a specimen must be removed before it can be infiltrated with wax. This process is commonly carried out by immersing specimens in a series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached. Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol. A series of increasing concentrations is used to avoid excessive distortion of the tissue.

The process of clearing was originally termed as such because the reagents used for this step have a high index of refraction and will render tissue transparent. Another important role of the clearing agent is to remove a substantial amount of fat from the tissue which otherwise presents a barrier to wax infiltration.

After dehydration and clearing, tissue must be infiltrated with the supporting medium. This medium, generally referred to as an embedding medium, holds cells and intercellular structures in their proper relationship while thin sections are cut. The most commonly used waxes for infiltration are the commercial paraffin waxes. A paraffin max is usually a mixture of straight chain or n-alkanes with a carbon chain length of between 20 and 40; the wax is a solid at room temperature but melts at temperatures up to about 65°C to 70°C. Paraffin wax can be purchased with melting points at different temperatures, the most common for histological use being about 56°C to 58°C, at this melting point it tends to be slightly viscous, but this decreases as the temperature is increased. The traditional advice with paraffin wax is to use this about 2°C above its melting point.

Protocol for Using EMS Rapid Pro Processing System

Special Notes

- Do not use sponge bags
- Precise control of temperature is critical to success.

The following is an example of solution and time for manually (hand processing) fresh tissue specimens that are about 2 mm - 4 mm thick *(For your convenience the individual EMS Catalog numbers are included for easy referencing. When ordering the complete ERPP Kit, use Catalog Number 26750-10)*:

EMS Catalog Number	Step and Solution Used	Time and Temperature
26750-01	1. ERPPS-1(10-12% Formalin)	3-min at 46°C (115°F)
26750-02	2. ERPPS-2 (70% ethanol)	3-min at 46°C (115°F)
26750-03	3. ERPPS-3 (95% ethanol)	3-min at 46°C (115°F)
26750-04	4. ERPPS-4 (98% ethanol)	3-min at 46°C (115°F)
26750-05	5. ERPPS-5 (90% Xylene)	3-min at 46°C (115°F)
26750-04	6. ERPPS-4 (98% ethanol)	3-min at 46°C (115°F)
26750-05	7. ERPPS-5 (90% Xylene)	3-min at 46°C (115°F)
	8. Paraffin	25 min at 60°C

Tissue Preparation

In this procedure, tissue is fixated, dehydrated through a series of graded ethanol baths to displace the water, and then infiltrated with wax. The infiltrated tissues are then embedded into wax blocks. Once the tissue is embedded, it is stable for many years.

Thickness: About 2 mm - 4 mm thick

Area: 20 mm × 30 mm

Fixed tissue: Cut large organs about 2mm-4 mm slices and store in ERPPS-1 solution for about 3 minutes.

Unfixed tissue: Slices of tissue should be fixed in ERPPS-1 solution for 3 minutes before processing.

Times: All times in processing fluids for this schedule are for about tissues 2 mm - 4 mm thick. Tissues thicker than that will require longer times.

Clearing agent: ERPPS-5 (Xylene) will clear tissues in similar times should be used.

Processing time: Approximately 21 minutes before paraffin embedding

Embedding Tissues in Paraffin Blocks

Tissues processed into paraffin will have wax in the cassettes; in order to create smooth wax blocks, the wax first needs to be melted away placing the entire cassette in 58°C paraffin bath for 15 minutes. Turn the heat block on to melt the paraffin one hour before adding the tissue cassettes.

Directions

1. Open cassette to view tissue sample and choose a mold that best corresponds to the size of the tissue. A margin of at least 2 mm of paraffin surrounding all sides of the tissue gives best cutting support. Discard cassette lid.
2. Put small amount of molten paraffin in mold, dispensing from paraffin reservoir.
3. Using warm forceps, transfer tissue into mold, placing cut side down, as it was placed in the cassette.
4. Transfer mold to cold plate, and gently press tissue flat. Paraffin will solidify in a thin layer which holds the tissue in position.
5. When the tissue is in the desired orientation add the labeled tissue cassette on top of the mold as a backing. Press firmly.
6. Hot paraffin is added to the mold from the paraffin dispenser. Be sure there is enough paraffin to cover the face of the plastic cassette.
7. If necessary, fill cassette with paraffin while cooling, keeping the mold full until solid.
8. Paraffin should solidify in 30 minutes. When the wax is completely cooled and hardened (30 minutes) the paraffin block can be easily popped out of the mold; the wax blocks should not stick. If the wax cracks or the tissues are not aligned well, simply melt them again and start over.

The tissue and paraffin attached to the cassette has formed a block, which is ready for sectioning. Tissue blocks can be stored at room temperature for years.

Sectioning Tissues

Tissues are sectioned using a microtome.

Directions

1. Turn on the water bath and check that the temp is 33-35*C. Blocks to be sectioned are placed face down on an ice block for 10 minutes.
2. Set the dial to cut 10 µM sections to order to plane the block; once it is cutting smoothly, set to 2-5 µM sections. The blade should angled at 5º.
3. Face the block by cutting it down to the desired tissue plane and discard the paraffin ribbon. If the block is ribboning well then cut another four sections and pick them up with forceps or a fine paint brush and float them on the surface of the 35*C water bath.
4. Float the sections onto the surface of clean glass slides. If the block is not ribboning well then place it back on the ice block to cool off firm up the wax. If the specimens fragment when placed on the water bath then it may be too hot.
5. Place the slides with paraffin sections on the warming block in a 65°C oven for 20 minutes (so the wax just starts to melt) to bond the tissue to the glass. Slides can be stored overnight at room temperature.

Electron
Microscopy
Sciences

For any questions or for ordering information,
please contact Customer Service at
1-800-523-5874

Thank you for choosing
Electron Microscopy Sciences!

www.emsdiasum.com
sgkcck@aol.com

Tel: 215-412-8400 ♦ *Fax:* 215-412-8450

Electron Microscopy Sciences
P.O. Box 550
1560 Industry Road, Hatfield, PA 19440