

HISTOLOGICAL PROCESSING: CLEARING

Principle

If the substance chosen to include the sample is paraffin, processing involves three steps: dehydration, clearing and infiltration (or impregnation), sequential steps to remove all the water that can be extracted from the tissue.

For the dehydration stage, alcohols are preferred, agents such as Xylene and / or its substituents such as Citrosol and Isoparaffin H (aromatic hydrocarbon mixture) are used for the clearing stage.

If Isoparaffin H is used instead of Xylene, no method changes are required, it is also possible to work with the same times for different stages of work. In addition, Isoparaffin H reacts with water or traces of water, more noticeably turbid than Xylene, this is related to the structure of Isoparaffin H. Therefore, we must pay attention not to use technical grade solvents together with Isoparaffin H.

Material

Histological samples

Reagents

- Clearing reagents:

Code	Description
251769	Xylene, mixture of isomers for clinical diagnosis (*)
255069	Isoparaffin H (Substitute of Xylene) for clinical diagnosis (*)
253139	Citrosol (Substitute of Xylene) for clinical diagnosis (*)

- Other reagents:

Code	Description
253211	Paraffin M.P. 56-58°C pellets for clinical diagnosis (*)
256993	Paraffin M.P. 55-58°C plasticized + DMSO pellets for clinical diagnosis (*)
252931	Formaldehyde 3.7-4.0% w/v buffered to pH=7 and stabilized with methanol for clinical diagnosis (*)
192695	Ethanol 70% v/v (BP) pharma grade
251085	Ethanol 96% v/v for clinical diagnosis (*)
251086	Ethanol absolute for clinical diagnosis (*)

Sample preparation

The samples are fixed in 4% formaldehyde solution, depending on their size and characteristics. Subsequently they are washed thoroughly in running tap water.

Technique

Histological processing: The samples, once fixed, are carefully dehydrated with alcohol. The alcohol is eliminated by treatment with alcohol soluble intermediates and paraffin. This ensures that the tissue is completely impregnated with paraffin, and after inclusion in a block, can be cut better.

Solution time

Stage	Baths	Processing time
Fixing	Formol	
Dehydration	Ethanol 70%	2 hours
	Ethanol 96 %	2 hours
	Ethanol absolute	2 hours
	Ethanol absolute	1 hour
	Ethanol absolute	1 hour
Clearing	Xylene/Citrosol/Isoparaffin H	1 hour
	Xylene/Citrosol/Isoparaffin H	1 hour
	Xylene/Citrosol/Isoparaffin H	1 hour
Inclusion	Paraffin	1 hour
	Paraffin	1 hour
	Paraffin	2 hours

The processing times in each bath are depending on the type and size of sample. Once the paraffin has solidified, the mold is separated from it, producing a paraffin block ready to cut into thin sections in a microtome. Samples included in paraffin (blocks) are stored in a refrigerated place, as this improves the cutting capacity. Heating the blade improves the cutting capacity.

Results

From the samples thus prepared, thin cuts are made with the microtome, called paraffin sections. The cuts are dewaxed, rehydrated and then stained, following the histological procedure appropriate to each case.

Technical note

The mode of use for the histological processor and the internal SOPs of the laboratory must be observed for each bath change. Observe the operating instructions of the devices and the maintenance instructions. Check the baths regularly. Change regularly; Their clouding or the perception of the smell of a clearing liquid in the last paraffin bath indicates the need to change them. Ensure that the optimum working temperature of the paraffin baths is maintained (4 ° C above the solidification point). Maintain the minimum adequate quality of solvents. Do not overfill the paraffin wax cassettes with sufficient paraffin.

Sample preparation

All samples should be treated according to the state of the technology. All samples must be unambiguously labeled.

Diagnostics

Diagnosis should be established only by authorized and qualified persons. Each application should involve appropriate controls to rule out erroneous results.

Storage

The product should be stored at room temperature.

Expiration

The product stored at room temperature and in a tightly closed container is usable up to the expiration date indicated on the package.

Notes on use

In order to avoid errors, the staining must be carried out by specialized personnel. For professional use only. The national directives on safety at work and quality assurance must be complied with.

Advise on disposal of waste

Solutions used and expired solutions should be disposed of as hazardous waste and local waste disposal regulations must be observed. If further questions are asked about disposal, they may be processed through E-Mail: info.es@itwreagents.com. Inside the EU are valid the requirements based on Council Directive 67/548 / EEC on the approximation of the laws, regulations and laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances in the relevant version.

Classification of hazardous substances

Observe the classification of dangerous substances on the label and the information on the safety data sheet.

Manufacturer

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(*) Sanitary product for In Vitro Diagnostics

