HISTOLOGICAL PROCESSING: FIXATIVES

Principle

Fixation is a procedure designed to stop all degradation processes that occur after obtaining the sample to be studied or after the death of the organism from which it is obtained the sample. The aim is to preserve the composition, structure and state of the tissue to the extent possible. It also avoids other processes such as autolysis and decomposition.

The fixing agent is selected according to: the kind of problem to be diagnosed, the type and size of the material, type of inclusion media, and the method of staining to be used.

- Formol:

Fixation typically occurs in 4% or 10% formaldehyde solutions. Can be used directly as working solution, the buffered formaldehyde solution 3.7-4.0% buffered to pH = 7 and stabilized with methanol for clinical diagnosis (cod. 252931), or if formalin is used at 37-38% w/w, it is diluted with water or with buffer solution, to reach the corresponding working concentration. Formol contains methanol to prevent polymerization of formaldehyde to paraformaldehyde.

- Boiun liquor:

Bouin's liquid is a solution formed by various substances with fixative properties (Formol, Prictic Acid and Acetic Acid). It is especially indicated for the fixation of soft tissues, normally is used to fix glands and reproductive organs. Formol alone is an excellent fixative agent, in fact it is the general-purpose fixative in most procedures and laboratories. The other two components modify the result that would be obtained if only fix with formalin, providing a lower hardness to the fixed sample, facilitating the correct fixation of the inner areas of the sample and reducing tissue contraction.

Material

- Formol:

Material of tissue samples and organs of human origin.

- Boiun liquor:

Sample can be washed with some buffer at physiological pH, to remove blood or any other foreign particle that we could have transferred to the sample during its extraction. Although the maximum thickness of the sample depends on the type of tissue, in general it can be said that it should not exceed 0.5-1cm. This ensures a fast entry of the fixative before the degradation of the sample.

Reagents

Code	Description
252931	Formaldehyde 3.7-4.0% w/v buffered to pH=7 and stabilized with methanol (CE-IVD) for clinical diagnosis (*)
253572	Formaldehyde 30-36% w/v concentrated buffered to pH=7 stabilized with methanol (CE-IVD) for clinical diagnosis (*)
256462	Histofix ® Preservative ready to use for clinical diagnosis (*)(1)
257462	Histofix ® Preservative ready to use (pink)(CE-IVD) for clinical diagnosis(*)(1)
258462	Histofix ®-Safe Preservative ready to use (CE-IVD) for clinical diagnosis (*)(1)
254102	Bouin Liquor for clinical diagnosis

Preparation of solutions

Formaldehyde 4% solution:

Mix 1 part of the Formaldehyde solution 30-36% w/v buffered concentrate at pH=7 stabilized with methanol with 9 parts of distilled water (1:10 dilution).

Procedure

The fixation of the samples should take place according to the size and the characteristics of the tissue.

In order to obtain an optimum fixation, this must be done as soon as possible after the extraction of the sample from the tissue. The penetration of formaldehyde into tissue is related to temperature.

- Formol:

  1. The pieces of tissue, after the shot, are introduced into formalin solution 3 - 4.0%.

  2. Place samples in a sufficiently wide container (to avoid spills and allow good handling) on a fixative volume of at least 20 times greater than that of the sample. The sample should be small in size if it is desired to be used for microscopic studies to ensure fast enough fixation of the innermost area. For the conservation of samples for the macroscopic study, the samples may be larger.

  3. Although not essential, constant and gentle agitation is recommended.

  4.Time of impregnation: it will depend on the size of the sample and the temperature (with heat the fixation is faster but of lower quality).

  5. In a refrigerated environment, the fixation is slower but the cold reduces the processes of degradation while fixation occurs. This is why it is usually done at room temperature or at 4ºC and adjust the setting time according to the nature of the sample and the chosen temperature.

  6. The fixing time is usually a few hours at room temperature and for small samples, and up to 12 hours or more, if the fixation is carried out at 4 ° C.

  7. Once the fixing process is finished, it is recommended to perform three washes of at least 5 minutes in running water.

- Boiun liquor

  1. Samples should be placed in a container with a sufficient volume of fixative solution in its interior, between 10 and 40 times greater than the volume of the sample. This ensures that the amount of fixative available will be sufficient for the entire process.

  2. The length of the fastening process must be adapted to the size and type of tissue by means of the experience, but as a general rule the fixing time is between 4 and 24 hours.

  3. It is very important to carry out washing after the fixing process, generally to wash all the fixing substances, but especially for picric acid. Insufficient washing will cause the action of picric acid is maintained over time even after the preparation is mounted, which will greatly diminish the effect of dyes of basic characteristics. Recommended 5 washed in alcohol 50% and 5 more in alcohol 70% until discoloration of the sample.

  4. If necessary, the sample can be kept in 70% alcohol until it can be processed.

Technical note

The equipment used should correspond to the requirements of a clinical diagnostic laboratory.

Preparation of samples

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

Diagnostics

Diagnostics should be established only by authorized and qualified persons. They must use terminology in force. Each application should involve adequate controls to discard wrong results.

Storage

The staining solution should be stored at room temperature.

Expiration

The product stored at room temperature and in a tightly closed container is usable up to the date of expiry date stated on the packaging.

Notes on use

To avoid errors, the technique must be carried out by qualified personnel. For professional use only. National occupational safety and quality assurance directives must be observed. Before use, consult safety data sheet.

Advise on disposal of waste

Used and expired solutions must be disposed of as hazardous waste in accordance with local waste disposal regulations. If you have any further questions about disposal or the procedure, please contact us by e-mail: info.es@itwreagents.com. Within the EU, the regulations based on Regulation (EC) No. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No. 1907/2006 on classification, packaging and labelling of dangerous substances, in its current version, apply.

Classification of hazardous substances

Take into account the classification of dangerous substances on the label and the indications in the safety data sheet.

Manufacturer

Panreac Química S.L.U.
An ITW Company
C / Garraf, 2 - Polígono Pla de la Bruguera
E-08211 Castellar del Vallès
(Barcelona, Spain
Tel. (+34) 937 489 400
Fax (+34) 937 489 401

(*) Sanitary product for In Vitro Diagnostics

IVD CE

(1) THIS PRODUCT IS A VARIABLE OF OUR FORMALDEHYDE 3.7 - 4.0% BUFFERED PH=7 AND STABILIZED WITH METANOL DC, REF. 252931, BUT IS SOLD WITH ANOTHER COMMERCIAL NAME OF OUR TRADEMARK FOR DIAGNOSIS IN VITRO.