

## Chloral haematoxylin solution technical information

Technical's card code 09-175

Product code 09-175

Stability of product properly conserved at 15-25°C 24 month.




Pack 500-1000 ml or on request

Produce in Italy by:

DDKItalia S.r.l

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in case of emergency UE number		112
in case of emergency UK number		999
en cas d'urgence Suisse		145

## Purpose

Haematoxylin and eosin staining protocol is used frequently in histology to examine thin sections of tissue. Haematoxylin stains cell nuclei blue, while eosin stains cytoplasm, connective tissue and other extracellular substances pink or red. Eosin is strongly absorbed by red blood cells, colouring them bright red. In a skilfully made H & E preparation the red blood cells are almost orange, and collagen and cytoplasm (especially muscle) acquire different shades of pink. When the staining is done automatically, the subtle differences in eosinophilia are often lost. Haematoxylin stains the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm and the matrix of hyaline cartilage) blue. In contrast, eosin stains the cytoplasm and collagen pink.

Chloral haematoxylin is intended for nuclear staining in histology and cytology. All haematoxylin solutions are reserved solely for use "in vitro". Haematoxylin, is a common nuclear staining, which is isolated from an extract of blue wood (Haematoxylon campechianum). Before using haematoxylin for nuclear staining, it is necessary to transform it by oxidizing hematein and combine it with a metal ion (mordant). The stains were most suitable aluminium salts or iron. Generally, they are classified as progressive haematoxylin or regressive based on the dye concentration. The progressive color: (for example, Mayer) has a lower concentration of dye and selectively stain the nuclear chromatin without affecting the cytoplasmic structures. The desired intensity is a function of time.

Regressive stains (ex, Harris haematoxylin) work intensively on all nuclear and cytoplasmic structures. To get the answer correct color, remove the excess dye from the tissue section. Gill's formulation is used as a progressive cytological staining, while the formulations can be used as Gill 2 color progressive or regressive, depending on the duration. These solutions are made from haematoxylin, treated with aluminium, and stabilized with glycols. The complex aluminium-hematein (positively charged) is combined with nuclear DNA phosphate groups (negatively charged) and takes on the characteristic blue color of haematoxylin purple.

## Method

Paraffin sections and bring water	
2. Place the sections in haematoxylin	1-10 minutes*
3. Wash in lukewarm water source (a color change)	3-20 minutes
4. Place the sections in eosin	1-3 minutes **
5. Rinse in tap water quickly	
6. Differentiate in 95 ° alcohol for a few moments	
7. Dehydrate in ethanol 95 °	30-40 second
8. Absolute ethyl alcohol	4-5 minutes
9. Absolute ethyl alcohol	4-5 minutes
10. Xylene	5-15 minutes
11. Xylene	5-15 minutes
12. Mount with DdMount (04-103)	

## Results:

Nuclei and basophilic material in blue-violet

	Eosin Y	Eosin B	Eosin purified
Cytoplasm, collagen elastin	red orange	red	red
Erythrocytes	yellow orange	red orange	red orange

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### Performance characteristics

The chromatin must appear between blue and black-blue, while the nucleoli should be clearly visible. The cytoplasmic staining with Gill 2 haematoxylin solutions are usually pale or absent, so the acid differentiation might not be necessary.

\* Times vary according to age, types of solutions, thickness of sections, et. When Gill (code 09-178) modified solution is used, get the best result, staining time (maximum 1-5 minutes), for best change in color, wash quickly in tap water, and then in Scott acidulated solution, (code 00-136). For sections fixed in Bouin, we recommend the use of haematoxylin modified acid AB (code 09-183).

\*\* Please note the alcoholic loses eosin stain with the use, of the days are stretched over time colouring. If you are using purified eosin, check the time, and possibly diluted in ethyl alcohol 96 ° C, if the cytoplasmic staining was too strong.

Before use, filter the following solutions; alcoholic eosin, eosin phloxine; Harris haematoxylin, Gill's haematoxylin. The acidified aqueous solution of eosin is prepared by slowly adding glacial acetic acid.

#### Technical note

Follow normal precautions for laboratory reagents. Dispose of waste according to regulations at the local, regional or national level. Refer to Data Sheet Material Safety Data for updated information on risks, hazards and safety associated with the use of these products.

\* Risk and Safety Statements outside the EU. The eosin solution in alcohol is flammable and harmful. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

\* Target organs: eyes and nerves.

Eosin in aqueous solution. Caution: substance not yet fully tested. Avoid contact and inhalation of the solution of Harris haematoxylin. Organs: heart and nerves. Solutions based haemallum are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing. Organs affected: liver and kidneys. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

\* Risk and Safety Statements (U.E.).

The eosin solution in alcohol is highly flammable and harmful. Highly flammable. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Eosin in aqueous solution. Caution: Substance not yet fully tested. Solution of haemallum. Do not breathe vapours. Avoid contact with skin and eyes. Gill haematoxylin Solutions are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing.

\* The microscope used should meet the requirements of a medical diagnostic laboratory. Carefully follow the instructions for the fixative. If an automated tool was used for staining, follow the instructions of the equipment and software. Remove surplus immersion oil before storing. Sample preparation. All samples must be treated according to the technology. All samples must be marked so as to be easily identified. Tools should be used for sampling and sample preparation, which must be observed strictly to manufacturer's instructions about the application and instructions. Diagnostic. The diagnosis should be performed only by authorized and trained persons. Valid nomenclatures must be used. Further tests must be selected and implemented according to recognized methods.

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\* Conservation. The staining solution should be stored at a temperature between +15°C to 20°C, the dye at +5°C to 30°C. Store at 4-6°C all kit containing silver solutions and Schiff reagents. The solution and dyes must be used before the expiration date. Stability. After first opening the bottle, the dye solution and the dyes are stable until the expiration date when stored at the temperature requested. Always keep the bottles tightly closed. Instructions for use. To avoid errors, the staining process must be performed by qualified personnel. For professional use only. Must observe the National guidelines for work safety and quality assurance. Microscopes are used according to the standard. Protection against infection. Must be taken with laboratory guidelines for the protection against infection. Instructions for disposal. The solutions used and those have expired must be disposed of as special waste according to local regulations regarding disposal of waste.

#### Endnotes

- 1 The timing suggested in the leaflet are approximate and may vary according to your specific needs. If they are used intensively, for staining solutions may lose their dyes, so it is necessary to extend the time of staining solutions, or replace with new products.
2. Include positive control slides in each session.
3. Some hydraulic systems deliver acidic water, unsuitable for use for the part of the procedure for the blue coloration. If tap water is acidic, instead using a dilute alkaline solution, for example, water buffered by Scott.
4. The presence of purple or red-brown nuclei a blue color indicates unsatisfactory.
5. If you over-eosin staining, nuclear staining may be masked. If done correctly, with eosin staining shows a three-tone effect. To increase the differentiation of eosin, extend the time of immersion in alcohol, or use a first alcohol with a higher water content. You can adjust the times of immersion in alcohol to obtain an adequate eosin staining.
6. We do not recommend the addition of stock solution in the working solutions of haematoxylin and eosin.
7. Avoid excessive drag (carryover) of water solutions in alcoholic eosin.
8. The data generated by this procedure are to be used only to support the diagnosis and should be evaluated in conjunction with other tests and diagnostic data

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