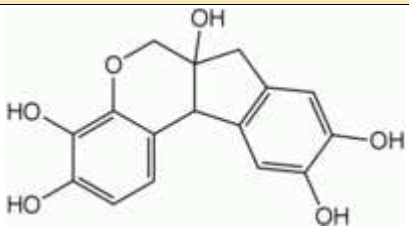
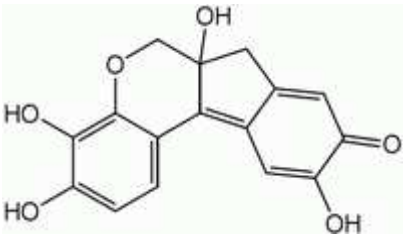
Haematoxylin

Haematoxylin	
	
IUPAC name	6,6a,7,11b-tetrahydroindeno [2,1-c]chromene-3,4,6a,8,9-pentaol
Identifiers	
CAS number	517-28-2
PubChem	10603
MeSH	Hematoxylin
Properties	
Molecular formula	$C_{16}H_{14}O_6$
Molar mass	302.279
Hazards	
Except where noted otherwise, data are given for materials in their standard state (at 25 °C, 100 kPa) Infobox disclaimer and references	



Haematein

Haematoxylin, **hematoxylin**, **Natural Black 1**, or **C.I. 75290** is extracted from the wood of the logwood tree. When oxidised it forms haematein, a compound with rich blue-purple colour, and is used, together with a suitable mordant (most commonly Fe(III) or Al(III) salts), to stain cell nuclei prior to examination under a microscope. Structures that stain with haematoxylin are called basophilic.

Its CAS number is [517-28-2] and its SMILES structure is OC(C(O)=C4)=C C1=C4CC3(O) C1C2=CC=C(O) C(O)=C2OC3.

Haematoxylin and eosin stain is one of the most commonly used stains in histology. It is a permanent stain as opposed to temporary stains (e.g. iodine solution in KI).

Other common stain is phosphotungstic acid haematoxylin, a mix of haematoxylin with phosphotungstic acid.

In 1970's, due to clear felling of forests in Brazil and Central America, there was a shortage of logwood and therefore of haematoxylin. Its price went to record heights, which affected the cost of diagnostic histopathology, and prompted a search for alternative nuclear stains. Before the use of any alternatives became firmly established, hematoxylin returned to the market, though at a higher price, and resumed its place in histopathology. There were several dyes recommended as replacements: Celestine blue B (CI 51050), Gallocyanin (CI 51030), Gallein (CI 45445) and Solochrome cyanin (CI 43820). All four used Fe(III) as the mordant. Another alternative is the red dye brazilin, which differs from hematoxylin by only one hydroxyl group.

Hematoxylin Staining Solutions

These stains are commonly employed for histologic studies. The mordants used to demonstrate nuclear and cytoplasmic structures are Alum and Iron, forming lakes or colored complexes (dye-mordant-tissue complexes), the color of which will depend on the salt used. Aluminum salt lakes are usually colored blue white while Ferric salt lakes are colored blue-black.

Aluminum Hematoxylin Solutions

The two main Alum Hematoxylin solutions employed are Ehrlich's Hematoxylin and Harris Hematoxylin. Alum Hematoxylin solutions impart on the nucleus a light transparent blue stain which rapidly turns red in the presence of an acid.

Alum or Potassium Aluminum Sulfate used as the mordant usually dissociates in an alkaline solution, combining with -OH of water to form insoluble Aluminum Hydroxide. In the presence of excess acid, Aluminum Hydroxide cannot be

formed thus failure of Aluminum Hematoxylin dye-lake to form, due to lack of –OH ions. Hence, acid solutions of Alum Hematoxylin become red. During staining Alum Hematoxylin stained sections are usually passed on to an alkaline solution (e.g. 1% Hydroxide) in order to neutralize the acid and free the OH group, to form an insoluble blue Aluminum Hematin-Tissue Lake. Such procedure is known as *Blueing*.

When tap water is not sufficiently alkaline, or is even acid and is unsatisfactory for Blueing Hematoxylin, Tap Water Substitute consisting of 33.5 g $NaHCO_4$ and 20 grams $MgSO_4$ in 1000 cc of water, with Thymol (to inhibit formation of molds), is used to accelerate blueing of thin paraffin sections. Use of very cold water slows down the process while warming accelerates it. In fact, the use of very cold water below 10°C for blueing sections may even produce pink artifact discolorations on the tissue.

Ehrlich's Hematoxylin

Formula:

- 1 g Hematoxylin
- 100 ml Absolute Ethanol
- 60 g Potassium Alum (Aluminum Potassium Sulfate)
- 100 ml Glycerin
- 100 ml Distilled water
- 10 ml Glacial Acetic Acid (HOAc)

First, Dissolve 1 g Hematoxylin in 100 ml of Absolute Ethanol with gentle heat. Second, dissolve the 60 g of Potassium Alum (Aluminum Potassium Sulfate) in 100 ml Distilled water with 100 ml Glycerin with gentle heating and agitation. Third, mix the two solutions and add 10 ml Glacial HOAc. Then Expose to the air and sunlight for several weeks or months in a flask lightly plugged with cotton. Shake the solution daily. Finally, transfer the solution in a well-stoppered bottle and store in a warm place.

Hematoxylin may be partially oxidized and the stain may be used by addition of 0.3 g Sodium Iodate. As Hematoxylin solutions becomes oxidized, the color of the solution will change from purplish to deep red, while the pungent odor of HOAc will be replaced by a pleasant vineous aroma. Glycerin acts as a stabilizer and retards evaporation of the solution. However, glycerin appears to slow down ripening and hence may be added 4 to 6 weeks after the initial preparation.

Ehrlich's Hematoxylin is generally used for regressive staining, differentiated with 1% HCl in 70% Acid-Alcohol until the nucleus is selectively stained. Mucopolysaccharide substance such as cartilage and cement lines of bones are also stained intensely blue. Staining time is usually 15 to 40 minutes.

Harris Hematoxylin

Formula:

- 1 g Hematoxylin
- 10 ml Absolute Ethanol
- 20 g Ammonium/Potassium Alum
- 190 ml Distilled water

- 0.5 g Mercuric Oxide (Red)
- 10 ml Glacial Acetic Acid (HOAc)

First, dissolve 1 g Hematoxylin in 10 ml of Absolute Ethanol with gentle heating. Second, dissolve 20 g of Ammonium or Potassium Alum in 190 ml Distilled water inside a 500 ml flask or beaker. Third, add Mercuric Oxide and plunge immediately into cold water for rapid cooling. Using a wide-mouth container or flask will prevent a violent explosion due to the liberation of Oxygen upon addition of Mercuric Oxide to the solution. The solution should assume a dark purple color upon addition of Mercuric Oxide. The addition of 4% Glacial HOAc will give a more precise nuclear staining. Finally, the solution is then filtered and transferred into a well-stoppered bottle, and may be used off-hand or stored since it remains stable for a long time.

Harris Hematoxylin is widely used for routine nuclear staining in Exfoliative Cytology and for staining of sex chromosomes. The usual staining time is 5 to 20 minutes.

Cole’s Hematoxylin

Cole's Hematoxylin is another Alum Hematoxylin solution recommended for routine purposes, especially used in sequence with Celestine Blue.

Formula:

- 1.5 g Hematoxylin
- 50 ml of 1% Iodine in 95% Alcohol
- 700 ml of Saturated Aq. Ammonium Alum
- 250 ml Distilled water

Dissolve 1.5 g Hematoxylin in warm 250 ml Distilled water, and mix with 50 ml Iodine solution. Add 700 ml Saturated Aq. Ammonium Alum then boil. Finally, cool and filter before use. Staining time is 10 minutes.

Mayer’s Hematoxylin

Mayer's Hematoxylin is more vigorous in action than Ehrlich's Solution, giving little or no staining of Mucopolysaccharides. It is used in Celestine Blue Hemalum method of Nuclear Staining.

Formula:

- 1 g Hematoxylin
- 0.2 g Sodium Iodate
- 50 g Potassium Alum
- 1 g Citric Acid
- 50 g Chloral Hydrate
- 1000 ml Distilled water

Allow Hematoxylin, Alum, Sodium iodate to dissolve in water overnight. Then add Chloral Hydrate and Citric Acid. Boil for 5 minutes and cool.

Iron Hematoxylin Solutions

Two main Iron Hematoxylin solutions are employed for routine work in the laboratory: Weigert's Solution, using Ferric Ammonium Chloride, and Heidenhain's Solution, using Ferric Ammonium Sulfate (Iron Alum) as mordants. Both are active oxidizing agents; hence, do not prolong in storage as a prepared fixative.

They can be applied to tissues fixed in virtually all fixatives, producing permanent stains, provided all Iron mordants have been wiped out. Tissues that have been stored in alcohol for years and which would ordinarily fail to stain, will normally take Iron Hematoxylin. Tissue structures are stained blackish or grayish, according to the extent of differentiation, producing minimal eyestrain; hence, making it useful for photomicrography.

Weigert's Hematoxylin

Weigert's Solution is the standard Iron Hematoxylin used in the laboratory, especially for demonstrating muscle fibers and connective tissues. It is particularly recommended when the preceding stains contain acid (e.g. Van Gieson's Stain containing Picric Acid) which decolorizes nuclei stained with Alum Hematoxylin.

Formula

- *Solution A:*

* 1 g Hematoxylin
* 100 ml Absolute Ethanol

- *Solution B:*

* 4 ml 30% Anhydrous Ferric Chloride
* 1 ml Concentrated HCl
* 100 ml Distilled water

Hematoxylin is dissolved in Alcohol with gentle heating, while $FeCl_3$, HCl and Water are mixed in a different container. Both solutions are stable and may be stored 6 weeks before use. Ferric Chloride is usually added to the staining solution just before use, by mixing equal parts of the two solutions, producing a deep black mixture. The working solution will remain active for 1 to 2 days.

Heidenhain’s Hematoxylin

Heidanhain's solution is a cytological stain recommended for regressive staining of thin sections. It is utilized for the demonsrtation of both nuclear and cytoplasmic inclusions such as chromatin, chromosomes and mitochondria. Voluntary muscle striations and myelin are also well stained.

Formula

- *Mordant Differentiator:*

* 2.5 g Ferric Ammonium Sulfate (Iron Alum)
* 100 ml Distilled water

- *Hematoxylin Stain:*

* 0.5 g Hematoxylin
* 10 ml 95% Ethanol
* 90 ml Distilled water

Clear violet crystal of Alum are used and dissolved in distilled water. Then, Hematoxylin is dissolved in Ethanol with water in another container. Allow to ripen for 4 to 5 weeks, and store in tightly stoppered bottles. The Mordant Differentiator is used separately during the process of staining, instead of being added to the solution.

Phosphotungstic Acid Hematoxylin (PTAH)

PTAH usually demonstrates structures in paraffin as well as celloidin and frozen sections. Staining time is usually 12 to 24 hours.

Formula

- 1 g Hematoxylin
- 20 g Phosphotungstic Acid
- 1000 ml Distilled water

Completely dissolve the solids in separate portions of distilled water. Then, add together and stand under the light to ripen for several weeks. Immediate ripening may be obtained by adding 0.177 g Potassium.

The color of the solution ranges from reddish-brown to purple, although this is not a reliable guide for the study of stained tissues. Nuclei, fibrin, muscle striations, myofibrils and fibroglia are colored blue while collagen, bone and cartilage take an orange-red or brownish red to deep brick-red stain.

Staining is usually progressive, hence, microscopic examination of the materials every hour is recommended. 95% Alcohol usually removes the red component of the stain, so that dehydration and rinsing of sections should be brief.