



Mayer Haematoxylin

IVD

In Vitro Diagnostic Medical Device

Stability: 2 years under recommended storage conditions.

Storage conditions: Store at 15 - 25 °C, protected from fire and sunlight. Packaging to be stored tightly closed.

Procedure duration: ~ 25 min.

Content: Reagents:

Mayer's haematoxylin staining solution.

Application: Haematoxylin & Eosin (H&E) staining is the most frequently used staining method in pathological histology. It was introduced in 1876 by Wissowzky.

N. B!: The clinical interpretation of any staining, or its absence, should be complemented by morphological studies and proper controls, and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

All reagents have been optimally prepared for use and require no mixing or diluting.

Basic stages of H&E staining:

- 1. Haematoxylin staining** - progressive or regressive due to laboratory requirements for nuclear morphology examination.
 - Recommendable: when new haematoxylin solution (haemalum) is received in the lab, a method for its application to be chosen due to current laboratory requirements.
 - A qualified and experienced histotechnologist should perform both protocols with the same outcome!
 - Progressive staining is more specific. Staining duration should be empirically determined due to current laboratory requirements. It also depends on the method of fixation, nature and properties of samples. Regressive staining stains cells cytoplasm and needs differentiation step!
 - Haematoxylin solutions basic disadvantage is that they are not resistant to strongly acid dyes.
 - Solution should be filtered before use – at least once a week when frequently used. Also when there is sediment or metallic - appearing material, precipitated on the surface of the solution.
- 2. Differentiation** – to be applied only when REGRESSIVE staining is used – for elimination of excess dye from sections until the desired outcome (staining) is reached. Acid alcohol (0.1-0.5%) is used.
- 3. Blueing** – different alkaline solutions with pH levels above 5 could be used. Basically a wash with running tap water for 10-15 min is sufficient. The acid haemalum solution should be neutralized to give blue coloration of nuclei and stained cytoplasm structures. As higher is the pH value of the blueing solution, as faster is the process. The use of too strong solutions may result in sections loss!
- 4. Eosin counterstaining** – could be with water or alcoholic Eosin Y solution. It's a weakly acid solution that stains basic proteins in cytoplasm in variety of pink shades. Normally duration is 1- 3 min. If there is longer staining in alcohol Eosin Y solution, the haematoxylin staining may weaken.
- 5. Dehydration, lightening, mounting and examination.**

Control sections: appendix, intestine etc. due to their histological properties – useful for determination of best protocol configuration for best outcomes (staining) to be achieved. Modifications should be applied about the haematoxylin staining and differentiation step.

Method:

1. PROGRESSIVE STAINING:

1. Deparaffinize and bring sections to water	
2. Stain in Mayer's Haematoxylin	5 - 10 min due to lab requirements
3. Rinse in distilled water	Fast
4. Bluish in running tap water	5-15 min
5. Counterstain in eosin	1-3 min due to lab requirements
6. Wash in running tap water	1-3 min
7. Dehydrate in 95% and 100% Ethanol, clear in Xylene, mount in resin (optional) and examine.	

2. REGRESSIVE STAINING:

1. Deparaffinize and bring sections to water	
2. Stain in Mayer's Haematoxylin	5 min or more due to laboratory requirements
3. Differentiate in acid alcohol (0.1-0.5%)	To be empirical determined
4. Bluish in running tap water	5-15 min
5. Counterstain in eosin	1-3 min due to lab requirements
6. Wash in running tap water	1-3 min
7. Dehydrate in 95% and 100% Ethanol, clear in Xylene, mount in resin (optional) and examine.	

Results: Nuclei – blue

Cytoplasm - pink

Note: Exhausted Haematoxylin and Eosin solutions – that do not produce satisfactory outcome despite the same procedure was maintained – should be replaced with fresh ones!

References:

1. Lillie RD, (1965) Histopathologic Technic and Practical Histochemistry, 3rd edition
2. Histochemical uses of haematoxylin – Review – Godwin Avwioro, April - June 2011, JPCS, Vol. 1
3. Haematoxylin and counterstains – Anthony E. Woods
4. www.stainsfile.info
5. Haematoxylin and Eosin staining: Oversights and insights – Gary W. Gill, DAKO Special stains, second edition.
6. DAKO

Manufacturer :Emmonya Biotech Ltd. ,Chelopech, Sofia Area, Bulgaria