Heidenhain铁苏木素染色试剂盒

货号： G4480
规格： 4×100mL
保存： 室温，避光保存，有效期至少 2 年。

产品组成：

名称	4×100mL	保存
试剂(A): Heidenhain Differentiation	2×100mL	室温，避光
试剂(B): Heidenhain 铁苏木素染色液	100mL	室温，避光
试剂(C):伊红复染液	100mL	室温，避光

产品介绍：

苏木素(Hematoxylin)和伊红(Eosin)联合染色简称HE染色，是病理学和组织学最常用的一种染色方法。苏木精为碱性天然染料，可使细胞核着色。细胞核内染色质的主要成分是DNA，在DNA的双螺旋结构中，两条核苷酸链上的磷酸基向外，使DNA双螺旋的外侧带负电荷，呈酸性，很容易被带正电荷的苏木精碱性染料以离子键或氢键结合而被染色。

Heidenhain铁苏木素染色试剂盒以硫酸铁铵作为氧化剂和分化剂，根据不同的分化程度可显示不同的结构。染色后所有成分均为黑色或深灰黑色，不同组织结构的苏木素着色可被Heidenhain分化液以不同的速度褪去，黑色褪去顺序依次为：线粒体、横纹肌、核染色质。

操作步骤：(*仅供参考*)

1. 组织固定，石蜡包埋，切片3-5μm。
2. 切片脱蜡至水
   ① 二甲苯作用2次，每次5～10min，无水乙醇作用2次，每次3～5min。
   ② 95%的乙醇 3～5min，90%的乙醇 3～5min，80%的乙醇 3～5min，蒸馏水浸洗 1～3min
3. 染色
   ① Heidenhain Differentiation媒染 1h，蒸馏水冲洗 5～10s (见注意事项1)；
   ② Heidenhain铁苏木素染色液染色 1h，蒸馏水冲洗 20～30s；
   ③ （可选）伊红复染液 2-4min；
   ④ Heidenhain Differentiation分化或用蒸馏水1:1稀释Heidenhain Differentiation分化，并与自来水冲洗交替进行，显微镜下观察分化程度(见注意事项2)，自来水冲洗 10min
4. 脱水、透明、封固
   ① 80%乙醇 10～20s，90%乙醇 10～20s，95%乙醇作用2次，每次1～2min。
   ② 无水乙醇作用2次，每次2～3min，二甲苯透明3次，每次2～3min，中性树脂封片。

染色结果：

线粒体、横纹肌、髓磷脂、染色质等呈灰黑色。

注意事项：

1. Heidenhain Differentiation媒染时间和Heidenhain铁苏木素染色液染色时间根据不同的固定液而异。一般情况下，媒染和染色时间控制在1h即可，参考时间为：福尔马林、Bouin固定液、Carnoy固定液1h，Helly、Zenker等重铬酸盐固定液3h，四氧化锇、Flemming固定液24h。
2. 显微镜下控制分化程度，直到出现所需观察的结构。若分化过度，可用苏木素重染相同时间并重新分化。亦可用蒸馏水2:1稀释Heidenhain Differentiation后再进行分化，以便更好控制分化程度。
3. 切片分化后应彻底冲洗洗掉所有分化液，否则组织易褪色。系列乙醇应经常更换新液。
4. 胞浆复染(伊红或橙黄G)可突出核染色质，尤其在显示染色体或有丝分裂更有效。
5. 切片脱蜡应尽量干净 冷冻切片染色时间尽量要短。
6. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Heidenhain's Iron-Hematoxylin Stain Kit

Cat: G4480
Size: 4×100mL
Storage: RT, avoid light, valid for 2 years.

Kit Components

Reagent	4×100mL	Storage
Reagent(A): Heidenhain Differentiation	2×100mL	RT, avoid light
Reagent(B): Iron-Hematoxylin Solution	100mL	RT, avoid light
Reagent(C):Eosin Solution（Optional）	100mL	RT, avoid light

Introduction

Hematoxylin-Eosin staining,which also named HE stain,is the most basic method of routine section staining in pathology.Hematoxylin is an alkaline natural dye, which can stain the nucleus. The main component of chromatin in the nucleus is DNA. In the double helix structure of DNA, the phosphate groups on the two nucleotide chains are outward, making the outer side of the double helix of DNA negatively charged and acidic. It is easy to dye with positively charged hematoxylin basic dye by ion bond or hydrogen bond.

Heidenhain's Iron-Hematoxylin Stain Kit use ammonium ferric sulfate as oxidant and differentiator, and can display different structures according to different differentiation degrees.After dyeing, all components are black or dark gray black. The hematoxylin staining of different tissue structure can be removed by Heidenhain differentiation solution at different speed, and the order of black color removal is: mitochondria, striated muscle, nuclear chromatin.

Protocol(*for reference only*)

1) Fix tissue blocks , embed in paraffin and section.
2) Dewaxing and hydration of paraffin slices:
   ① Dewax in xylene twice for 10mins each,Absolute ethanol twice for 2mins each.
   ② 95% ethanol for 2mins,80% ethanol for 2mins,70% ethanol for 2mins.
   ③ Distilled water for 2mins.
3) Mordantly stain with Heidenhain Differentiation for 1h,rinse with distilled water for 5-10s.(Refer Note 1)
4) Stain with Iron-Hematoxylin Solution for 1h,Wash with tap water for 20-30s.
5) (*Optional*)Re-dyeing with Eosin Solution for 2-4min.
6) Differentiate with Heidenhain Differentiation or 1:1 water diluent of Heidenhain Differentiation.Wash alternately with tap water,the degree of differentiation was observed under microscope. (Refer Note 2)
7) Dehydration, transparency and sealing.
   ① 80%ethanol for 10-20s, 90%ethanol for 10-20s
   ② 95%ethanol twice for 1min, Absolute ethanol twice for 1min.
   ③ Xylene twice for 1min.Seal with resinene and view under the microscope.

Result

Mitochondria, Striated Muscle, Myelin, Chromatin, etc	Dark Gray

Note

1. The time of mordant dyeing by Heidenhain Differentiation and the time of staining by Iron -Hematoxylin Solution acording to different fixative.Generally, the mordant dyeing and dyeing time can be controlled within 1h. For different fixatives, the reference time is: formalin, Bouin fixative, Carnoy fixative for 1h. Helly, Zenker and other dichromate fixative for 3h. osmium tetroxide, Flemming fixative for 24h.
2. Microscopically, the degree of differentiation is controlled until the desired structure appears.If the differentiation is excessive, hematoxylin can be used to re dye and re differentiate at the same time.In order to better control the degree of differentiation, we can also dilute Heidenhain differentiation with distilled water 2:1 before differentiation.
3. All the differentiation fluid should be washed thoroughly after the slice differentiation, otherwise the tissue is easy to fade.Slice dewaxing should be as clean as possible. Series ethanol should be replaced frequently,To prevent over staining,the dyeing time of frozen section must be short.
4. Re-dyeing the cytoplasmic (by Eosin or Orange G) can highlight nuclear chromatin, especially in the display of chromosomes or mitosis.
5. For your safety and health, please wear experimental clothes and disposable gloves.