Masson 三色染色试剂盒(固绿法)

货号： G1343
规格： 7×50mL/7×100mL
保存： 室温，避光保存，有效期 1 年。

产品组成：

名称		7×50mL	7×100mL	保存
试剂(A) :Weigert 铁苏木素染色液	A1:Weigert 染液 A	25mL	50mL	室温，避光
	A2:Weigert 染液 B	25mL	50mL	室温，避光
临用前，取 A1、A2 等量混合即为 Weigert 铁苏木素染色液，不宜提前配制。				
试剂(B):酸性乙醇分化液		50mL	100mL	室温
试剂(C): Masson 蓝化液		50mL	100mL	室温
试剂(D):丽春红品红染色液		50mL	100mL	室温，避光
试剂(E):弱酸溶液		50mL	100mL	室温
试剂(F):磷钼酸溶液		50mL	100mL	室温，避光
试剂(G):固绿染色液		50mL	100mL	室温，避光

产品介绍：

结缔组织狭义上是指其含有的三种纤维：胶原纤维、网状纤维、弹力纤维，而胶原纤维是分布最广、含量最多的一种纤维。Masson 三色染色又称马松染色，是结缔组织染色中最经典的一种方法，是胶原纤维染色权威而经典的技术方法。所谓三色染色通常是指染胞核和能选择性的显示胶原纤维和肌纤维。该法染色原理与阴离子染料分子的大小和组织的渗透有关：分子的大小由分子量来体现，小分子量易穿透结构致密、渗透性低的组织；而大分子量则只能进入结构疏松的、渗透性高的组织。然而，淡绿或苯胺蓝的分子量都很大，因此 Masson 染色后肌纤维呈红色，胶原纤维呈绿色(淡绿)或蓝色(苯胺蓝)，主要用于区分胶原纤维和肌纤维。

三色染色的特点：①染色稳定；②分化时间短，1-2s；③色彩清晰鲜艳；④适用范围广，适宜于组织的石蜡切片、冰冻切片等染色；⑤所染切片保存时间长且不易褪色。

自备材料：

固定液：选用甲醛升汞或甲醛盐溶液，系列乙醇，蒸馏水

操作步骤：(*仅供参考*)

1. 切片常规脱蜡至水，用配制好的 Weigert 铁苏木素染色 5-10min。
2. 用酸性乙醇分化液分化，水洗。
3. 用 Masson 蓝化液返蓝 3-5min，水洗。蒸馏水洗 1min。
4. 丽春红品红染色液染色 5-10min。
5. 在上述操作过程中按蒸馏水:弱酸溶液=2:1 比例配制弱酸工作液，用弱酸工作液洗 1min。
6. 磷钼酸溶液洗 1-2min。用配制好的弱酸工作液洗 1min。
7. 直接入固绿染色液中染色 1-2min。用配制好的弱酸工作液洗 1min。
8. 95%乙醇快速脱水。无水乙醇脱水 3 次，每次 5-10s。
9. 二甲苯透明 3 次，每次 1-2min。中性树胶封固。

染色结果：

胶原纤维/蛋白	绿色
胞浆、肌肉、红细胞	红色
细胞核	蓝褐色

注意事项：

1. 切片脱蜡应尽量干净。固定起着重要的作用，使用不同的固定液可延或缩短染色时间。

2. 取 A1、A2 等量混合即为 Weigert 铁苏木素染液，一般 24h 失去染色力。
3. 酸性乙醇分化时间应根据切片厚薄、组织的类别和新旧而定。
4. 弱酸溶液可使色彩更清晰鲜艳，如使用量大可自行配制 0.1-0.3%乙酸溶液予以替代。
5. 磷钼酸分化时要在镜下控制，分化到胶原纤维呈淡红色、纤维呈红色即可。分化时间根据染色深浅而定，一般 1-2min。
6. Masson 蓝化液亦可自行配制 Scott 促蓝液或 0.1-1%碳酸锂水溶液予以替代。

Masson's Trichrome Stain Kit (Fast Green FCF Method)

Cat: G1343
Size: 7×50mL/7×100mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent		7×50mL	7×100mL	Storage
Reagent(A) :Weigert Hematoxylin Dyeing Solution	A1:Weigert Stain Solution A	25mL	50mL	RT, avoid light
	A2:Weigert Stain Solution B	25mL	50mL	RT, avoid light
Before use, mix with A1 and A2 in equal amount to form the Weigert Hematoxylin Dyeing Solution. It is not suitable to prepare it in advance.				
Reagent(B): Acid Ethanol Differentiation Solution		50mL	100mL	RT
Reagent(C): Masson Bluing Solution		50mL	100mL	RT
Reagent(D): Ponceau Fuchsin Solution		50mL	100mL	RT, avoid light
Reagent(E): Weak Acid Solution		50mL	100mL	RT
Reagent(F): Phosphomolybdic Acid Solution		50mL	100mL	RT, avoid light
Reagent(G): Fast Green Solution		50mL	100mL	RT, avoid light

Introduction

In a narrow sense, connective tissue contains three kinds of fibers: collagen fibers, reticular fibers and elastic fibers. Collagen fibers are the most widely distributed and abundant fibers. Masson's Trichrome Staining is the most classical method in connective tissue staining. It is an authoritative and classical technique for collagen fibers staining. The Masson's Trichrome Staining usually refers to the staining of nucleus and the selective display of collagen and muscle fibers. The dyeing principle of this kit is related to the size of anionic dye molecules and the permeability of the tissues: the size of the molecules is reflected by the molecular weight, and the small molecular weight can easily penetrate the compact and low permeability tissues, while the large molecular weight can only enter the loose and high permeability tissues. However, the molecular weight of light green or aniline blue is very large, so after Masson's Trichrome Staining, muscle fibers are red, collagen fibers are green (light green) or blue (aniline blue), mainly used to distinguish collagen fibers from muscle fibers.

The characteristics of Masson's Trichrome Staining are: (1) stable dyeing; (2) short differentiation time, 1-2 s; (3) clear and bright color; (4) wide application, suitable for tissue paraffin section, frozen section and other dyeing; (5) long preservation time and not easy to fade.

Self Provided Materias

Fixative:10% formalin or 10% Neutral formalin., Series of ethanol, Distilled water.

Protocol(*for reference only*)

1. Dewax paraffin sections and rehydrate in graded alcohol.
2. Stain with Weigert Hematoxylin Dyeing Solution for 5-10mins.
3. Differentiat by Acid Ethanol Differentiation Solution for 1-2 seconds, then wash with water.
4. Bluing by Masson Bluing Solution for 3-5mins, then wash with water.Wash with distilled water for 1min.
5. Stain with Ponceau Fuchsin Solution for 5-10mins.
6. In the above operation process, mix distilled water and Weak Acid Solution with the radio of 2:1 to form Weak Acid Working Solution . Wash with Weak Acid Working Solution for 1min.
7. Wash with Phosphomolybdic Acid Solution for 1-2mins.Wash with Weak Acid Working Solution for 1min.
8. Stain with Fast Green Solution for 1-2mins.Wash with Weak Acid Working Solution for 1min.
9. Quick dehydration of 95% ethanol. Dehydrated by Anhydrous ethanol for three times, each time for 5 to 10 s.
10. Transparentize by xylene for three times and each time for 1-2mins. Seal with resinene.

Result

Collagen Fiber/Protein	Green
Cytoplasm, Muscle And Red Blood Cell	Red
Nucleus	Bluish Brown

Note

1. Slice dewaxing should be as clean as possible. Fixation plays an important role, and dyeing time can be prolonged or shortened by using different fixatives.
2. Weigert Hematoxylin Dyeing Solution is obtained by mixing A1 and A2 equally. It is generally recommended to be used within 4 hours.
3. The differentiation time of Acid Ethanol Differentiation Solution should be determined by the thickness of sections, the type of tissues and the old and new ones.
4. Weak Acid Solution can make the color clearer and brighter. If use in large quantities, 0.1-0.3% acetic acid solution can be prepared to replace it.
5. The differentiation of Phosphomolybdic Acid Solution should be controlled under the microscope until that the collagen fibers are light red and the fibers are red. Differentiation time depends on the depth of staining, generally for 1-2mins.
6. Masson Bluing Solution can also be replaced by Scott Blue Promoting Solution or 0.1-1% Lithium Carbonate Aqueous Solution.