Masson 三色染色试剂盒

货号： G1340
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
临用时，取 A1、A2 等量混合，成为 Weigert 铁苏木素染色液，不可预先配制后放置。				
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): 苯胺蓝染色液		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很大，因此 Masson 染色后肌纤维呈红色，胶原纤维呈绿色或蓝色，主要用于区分胶原纤维和肌纤维。

Masson 三色染色试剂盒的特点：◆染色稳定；◆分化时间短，1-2 秒；◆色彩清楚鲜艳；◆使用范围广，适宜于组织的石蜡切片、冰冻切片等染色；◆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、蒸馏水、系列乙醇、二甲苯、染缸

操作步骤：(*仅供参考*)

一、石蜡切片：

1. 切片常规脱蜡至水。
2. 用配制好的 Weigert 铁苏木素染色液染色 5min-10min。
3. 酸性乙醇分化液分化 5-15s，水洗。
4. Masson 蓝化液返蓝 3-5min，水洗。蒸馏水洗 1min。
5. 丽春红品红染色液染色 5-10min。
6. 在上述操作过程中按蒸馏水：弱酸溶液=2:1 比例配置弱酸工作液，用弱酸工作液洗 1min。
7. 磷钼酸溶液洗 1-2min。用配置好的弱酸工作液洗 1min。
8. 直接放入苯胺蓝染色液中染色 1-2min。用配置好的弱酸工作液洗 1min。
9. 95%乙醇快速脱水 2-3s，无水乙醇脱水 3 次，每次 5-10s。
10. 二甲苯透明 3 次，每次 1-2min，中性树胶封固。

二、冰冻切片

1. 切片在 10%的福尔马林溶液中固定 1 小时。
2. 用 Bouins 固定液室温过夜处理。
3. 后续操作同石蜡切片第 2-15 步。

染色结果：

细胞核、胶原纤维/蛋白	蓝色
细胞浆、肌肉、红细胞	红色

注意事项：

1. 切片脱蜡应尽量干净。
2. 取 A1、A2 等量混合，成为 Weigert 铁苏木素染色液，一般 24h 失去染色能力。
3. 组织固定起着非常重要的作用，使用不同的固定液可延长或缩短染色时间。
4. 经典 Masson 三色染色中，用 Harris 苏木精染核，但 Harris 苏木精染核后切片颜色不够鲜艳，本染液采用 Weigert 苏木素染细胞核，因为染色的目的主要在于区分胶原纤维和肌纤维，一般也可以省略该染色步骤。
5. 酸性乙醇分化时间应该依据切片薄厚，组织的类别和新旧而定。
6. 弱酸溶液可使色彩更清晰鲜艳，如使用量大可自行配置 0.1-0.3%乙酸溶液予以替代。若苯胺蓝过染，可省略第 6 步和第 7 步的弱酸清洗步骤，磷钼酸清洗后直接进行苯胺蓝染色。
7. 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。
8. 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

Cat: G1340
Size: 7×50mL/7×100mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent		7×50mL	7×100mL	Storage
Reagent (A):Weigert's Iron Hematoxylin Solution	A1:Weigert's Solution A	25mL	50mL	RT, avoid light
	A2:Weigert's Solution B	25mL	50mL	RT, avoid light
Mix equal parts of A1 and A2 to form Weigert's Iron Hematoxylin Solution,which is stable for about 4 h.				
Reagent (B): Acid Alcohol Differentiation Solution		50mL	100mL	RT
Reagent (C): Bluing Solution		50mL	100mL	RT
Reagent (D): Ponceau-Acid Fuchsin Solution		50mL	100mL	RT, avoid light
Reagent (E): Acetic Acid Solution		50mL	100mL	RT
Reagent (F): Phosphmolybic Acid Solution		50mL	100mL	RT, avoid light
Reagent (G): Aniline Blue Solution		50mL	100mL	RT, avoid light

Introduction

Masson Trichrome Stain Kit is intended for use in the study of connective tissue, muscle and collagen fibers.It is mainly used in distinguishing collagen from smooth muscle since these two component look similar under the microscope.In the Masson's Trichrome Stain Kit nuclei are stained with Weigert's iron hematoxylin, and cytoplasm and muscle are then stained with Beibrich scarlet-acid fuchsin. After treatment with phosphotungstic and phosphomolybdic acid, collagen is demonstrated by staining with aniline blue. Rinsing in acetic acid after staining renders the shades of color more delicate and transparent.

This kit may be used on formalin-fixed, paraffin-embedded or frozen sections.

Protocol(*for reference only*)

1. Dewax to distilled water.
2. Stain with Weigert's Iron Hematoxylin Solution for 5-10mins.
3. Differentiate with Acid Alcohol Differentiation Solution for 10-15s.
4. Blue in Bluing Solution for 2-5mins.Rinse in deionized water.
5. Stain with Ponceau-Acid Fucshin Solution for 5-10mins.Rinse in deionized water.
6. Differentiate in Phosphomolybic Acid Solution for 1-2mins or till collagen is not red.
7. Without rinsing, add Aniline Blue Solution to section and stain for 1-2mins.
8. Place section in Acetic Acid Working Solution(Mix 1 part of Acetic Acid solution and 2 part deionized water)for 1 min.
9. Dehydrate very quickly in 95% ethanol, absolute ethanol (these steps will wipe off Biebrich Scarlet-Acid Fuchsin Staining) and transparent in xylene.
10. Seal with resinene.

Result

Nucleus	Black
Cytoplasm, Muscle fibers	Red
Collagen	Blue

Note

1. Slice dewaxing should be as clean as possible.
2. Equal amount of A1 and A2 were mixed to form Weigert iron hematoxylin staining solution, which generally lost its dyeing ability within 24 h.
3. Tissue fixation plays a very important role. Different fixatives can prolong or shorten the dyeing time.
4. In the classic Masson trichromatic staining, Harris hematoxylin was used to stain the nucleus, but the color of the section after Harris hematoxylin staining was not bright enough. Weigert hematoxylin was used to stain the nucleus in this staining solution, because the purpose of staining is mainly to distinguish collagen fibers and muscle fibers, and this staining step can also be omitted generally.

5. The differentiation time of acid ethanol should be determined according to the thickness of slice, the type of tissue and the old and new.
6. Weak acid solution can make the color clearer and brighter. If it is used in a large amount, it can be replaced by 0.1-0.3% acetic acid solution. If aniline blue is over dyed, the weak acid cleaning steps in steps 6 and 7 can be omitted, and aniline blue dyeing can be carried out directly after phosphomolybdic acid cleaning.
7. The differentiation of phosphomolybdic acid should be controlled under microscope until the collagen fibers are light red and the fibers are red. The differentiation time depends on the depth of staining, generally 1-2mins.
8. Masson Bluing Solution can also be replaced by Scott bluing solution or 0.1-1% lithium carbonate solution.