

## 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。固绿分子量较大, 因此 Masson 三色染色(固绿法)染色后肌纤维呈红色, 胶原纤维呈绿色(固绿), 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; ◆容错率高, 能在较大染色时间跨度内取得令人满意的结果; ◆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: 选用中性甲醛盐溶液, 系列乙醇, 蒸馏水

### 操作步骤: (仅供参考)

#### 一、石蜡切片:

- 切片推荐厚度 3-8 $\mu$ m, 石蜡切片常规脱蜡至蒸馏水。
- (可选) 临用前 1: 1 混合试剂 A1 和 A2 配制 Weigert 铁苏木素染色液, 滴加覆盖切片染色 5-10min。
- (可选) 蒸馏水洗去多余染色液, 滴加酸性分化液分化 5-15s, 蒸馏水洗 30s。
- (可选) 滴加 Masson 蓝化液返蓝 3-5min, 蒸馏水洗 30s。
- 滴加丽春红品红染色液染色 5-10min。
- 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, 滴加弱酸工作液洗 30s。
- 倾去多余液体, 滴加磷钼酸溶液处理 1-2min。滴加弱酸工作液洗 30s。
- 倾去多余液体, 滴加固绿染色液染色 1-2min (见注意事项 11)。滴加弱酸工作液洗 30s。
- 95%乙醇快速脱水 2-3s, 无水乙醇脱水 2 次, 每次 5-10s。
- 二甲苯透明 2 次, 每次 1-2min, 中性树胶封固。

#### 二、冰冻切片

- 切片推荐厚度 8-12 $\mu$ m, 冰冻切片复温复水后用 Bouin 固定液室温过夜处理。(见注意事项 3)
- 75%乙醇清洗切片至黄色完全褪去。
- 后续操作同石蜡切片第 2-10 步。(见注意事项 4)





## 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，避免着色不均。本染色原理与结构有关，须避免因组织切片过薄导致的不上色和组织切片过厚导致的整体颜色发暗，因此推荐切片厚度 3-12 $\mu\text{m}$ 。
2. 组织类型和固定液选择对染色结果有很大影响，因此不同样本染色建议取少量切片进行预实验。
3. 如无 Bouin 固定液也可使用 G2310-Carnoy 固定液II代替进行过夜处理，使用 Carnoy 固定液II过夜处理后 75%乙醇浸洗 2 次，每次 1min 后即可正常染色。
4. 冰冻切片由于细胞成分保留较完整，更容易吸附染料，因此建议使用弱酸工作液将固绿染色液稀释 2-4 倍后染色 0.5-1min，弱酸工作液洗 30s 来避免过度蓝染。
5. 本染液采用 Weigert 铁苏木素染细胞核，与常规苏木素核染色结果不一致属正常现象，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。
6. 酸性分化液分化时间应该依据切片薄厚，组织的类别和新旧而定。
7. Masson 蓝化液亦可自行购买 G1865-Scott 促蓝液或配制 0.05-1%碳酸锂水溶液予以替代。
8. 弱酸溶液可使色彩更清晰鲜艳，如使用量大可购买 G2940-弱酸溶液或自行配置 0.5-1%乙酸溶液予以替代。若固绿过染，可将第 7 步的弱酸清洗步骤改为蒸馏水清洗。
9. 磷钼酸溶液易失效变色，正常呈淡黄色，如使用时已呈黄绿或发蓝建议弃用。
10. 本试剂盒内除试剂 A、F 外试剂均可循环使用，循环次数按照 0.8-1ml/切片计算。
11. 固绿染色时间根据组织胶原纤维多少自行调整，通常含胶原纤维多的组织上色较快，反之则上色较慢需延长染色时间。
12. 为了您的安全和健康，请穿实验服并戴一次性手套操作。



## 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 Fast Green is very large, so after Masson's Trichrome Staining, muscle fibers are red, collagen fibers are green (Fast Green), mainly used to distinguish collagen fibers from muscle fibers.

The characteristics of the Masson's Trichrome Stain Kit include: ◆ Stable staining; ◆ High fault tolerance, able to achieve satisfactory results within a large dyeing time span; ◆ Clear and bright colors; ◆ Widely used, suitable for staining tissues such as paraffin sections and frozen sections; ◆ The stained slices are stored for a long time and are not easily faded.

### Self Provided Materials

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

### Protocol( for reference only)

#### For Paraffin Section

1. The recommended tissue thickness is 3-8μm. Dewax to distilled water.
2. (Optional)Before use, mix A1 with A2 in equal amount to prepare Weigert's Iron Hematoxylin Solution, stain with Weigert's Iron Hematoxylin Solution for 5-10mins.
3. (Optional)wash with distilled water to remove excess stain, differentiate with Acid Differentiation for 5-10s, wash with distilled water for 30s.
4. (Optional)Blue in Bluing Solution for 3-5mins. Rinse in distilled water for 30s.
5. Stain with Ponceau-Acid Fuchsin Solution for 5-10mins.
6. In the above step, mix distilled water with Weak Acid Solution in 2:1 ratio to prepare Weak Acid Working Solution. Rinse the section with Weak Acid Working Solution for 30s.
7. Discard the excess solution., differentiate in Phosphomolybdic Acid Solution for 1-2mins. Rinse the section with Weak Acid Working Solution for 30s.
8. Discard the excess solution, stain with Fast Green Solution for 1-2mins(See Note 11). Rinse the section with Weak Acid Working Solution for 30s.
9. Dehydrate quickly in 95% ethanol for 2-3s, then dehydrate with absolute ethanol twice for each time 5-10s.





10. Transparent in xylene for twice, each for 1-2min, seal with resinene.

#### For Frozen Section

1. The recommended tissue thickness is 8-12 $\mu$ m. After thawing and rehydrating the frozen sections, treat overnight with Bouin fixative at room temperature. (See Note 3)
2. Wash the slices with 75% ethanol until the yellow color completely fades away.
3. The subsequent operation is the same as steps 2-10 of paraffin section. (See Note 4)

#### 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 to avoid uneven color. The principle of this staining is related to the structure, and it is necessary to avoid non coloring caused by too thin tissue slices and overall darkening caused by too thick tissue slices. Therefore, it is recommended to have a slice thickness of 3-12 $\mu$ m.
2. The type of tissue and the choice of fixative have a significant impact on the staining results, so it is recommended to take a small number of sections for pre experiments for staining different samples.
3. If there is no Bouin fixative, G2310-Carnoy fixative II can be used instead for overnight treatment. After overnight treatment with Carnoy fixative II, soak twice in 75% ethanol and each for 1min, then conduct normal staining.
4. Frozen sections are more prone to dye adsorption due to the complete retention of cell components. Therefore, it is recommended to use Weak Acid Working Solution to dilute Fast Green Solution 2-4 times and stain for 0.5-1 minutes, followed by washing with Weak Acid Working Solution for 30s to avoid excessive blue staining.
5. This staining solution uses Weigert iron hematoxylin to stain the nucleus, which is a normal phenomenon that is inconsistent with the results of conventional hematoxylin staining. The purpose of staining is mainly to distinguish between collagen fibers and muscle fibers, and this staining step can generally be omitted.
6. The differentiation time of Acid Differentiation Solution should be determined according to the thickness of slice, the type of tissue and the old and new.
7. Masson Bluing Solution can also be replaced by G1865-Scott Bluing Solution or G1840/G1841-Lithium Carbonate Solution, 0.05%/1%.
8. Weak Acid Working Solution can make the color clearer and brighter. If it is used in a large amount, you can buy G2940-Weak Acid Solution or prepare 0.5-1% acetic acid solution to instead of Weak Acid Working Solution. If Fast Green is over dyed, you can replace the step "Rinse the section with Weak Acid Working Solution for 30s" by "Rinse the section with distilled water for 30s"
9. Phosphomolybdic Acid Solution is prone to failure and discoloration, and normally appears light yellow. If it has turned yellow green or blue during use, it is recommended to discard it.
10. Except for Reagent A and F, all reagents in this kit can be recycled for use, with a cycle count of 0.8-1ml for each section.
11. The time for Fast Green staining is adjusted according to the amount of collagen fibers in the tissue. Generally, tissues with more collagen fibers tend to stain faster, while tissues with less collagen fibers tend to stain slower and require longer staining time.
12. For your safety and health, please wear laboratory clothes and disposable gloves for operation.

