V02

改良 Gomori 三色染色试剂盒

货号：G3510
规格：4×50mL
保存：室温，避光保存，有效期 6 个月。

产品组成：

名称		4×50mL	保存
试剂(A)：Weigert 铁苏木素	A1: Weigert 染液 A	25mL	室温，避光
	A2: Weigert 染液 B	25mL	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): 酸性分化液		50mL	室温
试剂(C): Gomori 染色液		50mL	室温，避光
试剂(D): Gomori 分化液		50mL	室温

产品介绍：

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。Gomori 染色液采用媒染剂和促染剂同时染色，可使结缔组织中的多种成分着色。

改良 Gomori 三色染色试剂盒可将胶原纤维染成淡绿色，肌纤维染成深绿色，细胞核染成蓝色。

操作步骤：（*仅供参考*）

1. 新鲜肌肉组织取材后立即进行冰冻切片，切片厚度为 10-15μm。
2. 滴加配制好的 Weigert 铁苏木素染色 5-10 min。
3. 流水冲洗 1-2min，镜下观察。如果染色过深，可用酸性分化液分化数秒（镜下观察）。
4. 自来水洗 10min 返蓝，蒸馏水洗 2-3 次。
5. 入 Gomori 染色液浸染 20- 40min，流水冲洗。
6. 在上述操作过程中按蒸馏水：Gomori 分化液=4:1 比例配制 Gomori 分化工作液。
7. 滴加 Gomori 分化工作液分化 30s-90s，以镜下观察分化适当为宜，流水冲洗。
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	亮绿色
肌纤维	青绿色
异常病变的肌纤维（包含体物质）	红色
线粒体	红色
细胞核	紫色
背景	绿色

注意事项：

1. Weigert 铁苏木素染液即配即用，一般 24h 失去染色力。
2. 组织要绝对新鲜，取材后要立即进行低温急冻，否则会形成冰晶，致使组织结构离散。
3. 采用 Weigert 染细胞核，因为染色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。
4. 酸性乙醇的分化时间应根据切片厚薄、组织的类别和新旧而定。

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5. Gomori 染色液应于冰箱内保存，超过有效期应丢弃以保证染色鲜亮。
6. Gomori 分化工作液分化的时间应根据染色液的效期长短做适当调整，一般新配置染色液，分化时间越长，染色液配置时间越长，分化时间越短，同时应根据实际染色情况做适当调整。
7. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

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Modified Gomori Trichromatic Stain Kit

Cat: G3510
Size: 4×50mL
Storage: RT, avoid light, valid for 6 months.

Kit components

Reagent		4×50mL	Storage
Reagent(A) : Weigert Iron Hematoxylin Solution	A1: Weigert Hematoxylin Solution A	25mL	RT, avoid light
	A2: Weigert Hematoxylin Solution B	25mL	RT, avoid light
Before use, mix A1 with A2 in equal amount to prepare Weigert Iron Hematoxylin Solution, it is not recommended to prepare in advance.			
Reagent(B): Acid Differentiation Solution		50mL	RT
Reagent(C): Gomori Staining Solution		50mL	RT, avoid light
Reagent(D): Gomori Differentiation Solution		50mL	RT

Introduction

Masson trichrome staining, also known as Masson staining, is the most classic method of connective tissue staining. It is an authoritative and classic technical method of collagen fiber staining. The so-called trichromatic staining usually refers to staining the nucleus and selectively displaying collagen fibers and muscle fibers. The dyeing principle of this method is related to the molecular size of anionic dyes and the permeability of tissues: the molecular size is reflected by the molecular weight, and the small molecular weight is easy to penetrate the tissues with dense structure and low permeability; The high molecular weight can only enter the loose structure and high permeability tissue. This kit uses mordant and dye promoter to dye at the same time, which can color a variety of components in connective tissue.

The kit can dye collagen fibers light green, muscle fibers dark green and nucleus blue.

Protocols*(for reference only)*

1. Pick fresh muscle tissue, immediately make frozen sections and cut the section in the thickness of 10-15 μm. (*see note 2*)
2. Drop with prepared Weigert Iron Hematoxylin Solution for 5-10 min.
3. Rinse with running water for 1-2min and observe under microscope. If the staining is too deep, it can be differentiated with Acid Differentiation Solution for several seconds (observe under microscope).
4. Blue with tap water for 10min and wash with distilled water for 2-3 times.
5. Dye with Gomori Staining Solution for 20-40min and rinse with running water.
6. During the above operations, prepare Gomori Differentiation Working Solution according to the ratio of distilled water: Gomori Differentiation Solution = 4:1.
7. Drop with Gomori Differentiation Working Solution for 30s-90s. Control the appropriate differentiation under the microscope, and rinse with running water.
8. Dehydrate in 95% ethanol rapidly and anhydrous ethanol for 3 times, 5-10s each time.
9. Transparent by xylene is for 3 times, 1-2min each time, and seal with resinene.

Result

Collagen fiber	Bright green
Muscle fiber	Turquoise
Abnormal muscle fibers (including body material)	Red
Mitochondrion	Red
Nucleus	Purple
Background	Green

Note

1. Weigert Iron Hematoxylin Solution is prepared and used immediately, and the dyeing effect is generally lost within 24 hours.
2. The tissue should be absolutely fresh. After taking the material, it should be frozen immediately at low temperature, otherwise ice crystals will be formed, resulting in the dispersion of tissue structure.

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3. The nucleus is stained with Weigert Iron Hematoxylin Solution, because the purpose of staining was mainly to distinguish collagen fibers from muscle fibers. Generally, this staining step can also be omitted.
4. The differentiation time of Acid Differentiation Solution should be determined according to the thickness of slices, the type of tissue and the preserving time.
5. Gomori Staining Solution shall be stored in the refrigerator and discarded beyond the validity period to ensure bright dyeing.
6. The differentiation time of Gomori Differentiation Working Solution should be properly adjusted according to the validity period of the staining solution. Generally, the longer the differentiation time, the longer the staining solution configuration time, and the shorter the differentiation time. At the same time, it should be properly adjusted according to the actual dyeing situation.
7. For your safety and health, please wear experimental clothes and disposable gloves.