V02

改良 Masson 三色染色试剂盒

货号：G1346
规格：8×50mL/8×100mL
保存：2-8℃，避光保存，有效期 1 年。

产品组成：

名称	8×50mL	8×100mL	保存
试剂(A)：媒染液(3×)	50mL	100mL	室温，避光
试剂(B)：天青石蓝染色液	50mL	100mL	2-8℃，避光
试剂(C)：Mayer 苏木素染色液	50mL	100mL	2-8℃，避光
试剂(D)：酸性分化液	50mL	100mL	室温
试剂(E)：丽春红品红染色液	50mL	100mL	室温，避光
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
试剂(H)：弱酸溶液(3×)	50mL	100mL	室温

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，淡绿或苯胺蓝染料的分子量很大，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。

改良 Masson 三色染色与常规 Masson 三色染色的区别在于采用天青石蓝苏木素淡染细胞核。其特点在于：分化时间短；色彩清晰鲜艳；适用范围广，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。改良 Masson 染色胶原纤维呈蓝色，肌纤维、胞质、纤维素、角蛋白和红细胞呈红色，细胞核呈蓝色，主要用于区分胶原纤维和肌纤维。

自备材料：

G2161-中性福尔马林固定液或 P1110-组织细胞固定液、Bouin 固定液、蒸馏水、系列乙醇、二甲苯或 G2150-环保组织透明脱蜡液、染缸

操作步骤：*（仅供参考）*

染色前，按照所需用量，将试剂(A)：媒染液(3×)和蒸馏水按照 1:2 比例混合即为媒染液(1×)；将试剂(H)：弱酸溶液(3×)和蒸馏水按照 1:2 比例混合即为弱酸工作液。

对于石蜡切片

1. 组织固定于 10%中性福尔马林或 4%多聚甲醛中，常规脱水包埋。
2. 推荐切片 3-6μm，常规脱蜡至蒸馏水。
3. 切片入媒染液(1×)浸染，于室温作用 8-12h 或置入 57℃-60℃的温箱内 1h 进行媒染，然后蒸馏水浸洗 3 次，每次 3min。（*见注意事项 2*）
4. 天青石蓝染色液滴染 2~3min，蒸馏水洗 2 次，每次 10-15s。
5. Mayer 苏木素染色液滴染 2~3min，蒸馏水洗 2 次，每次 10-15s。
6. 酸性分化液分化数秒，蒸馏水洗终止分化，自来水冲洗 10min（返蓝）。（*见注意事项 2*）
7. 丽春红品红染色液滴染 5-10min，蒸馏水洗 2 次，每次 10-15s。
8. 磷钼酸溶液分化处理 5-10min。
9. 倾去上液，切片不用水洗，直接滴加苯胺蓝染色液染 3-5min。
10. 用弱酸工作液洗去苯胺蓝溶液后，继续滴加弱酸工作液覆盖切片处理 2min。

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11. 95%乙醇快速脱水 3-5s。 无水乙醇脱水 2 次，每次 5-10s。 （*见注意事项 6*）
12. 二甲苯透明 2 次，每次 1-2min。 中性树胶封固。

对于冰冻切片

1. 组织固定于 10%中性福尔马林或 4%多聚甲醛中，梯度蔗糖脱水 OCT 包埋制作冰冻切片。
2. 推荐切片 8-15μm，染色前切片置于蒸馏水中复温 3min。
3. 染色步骤同石蜡切片步骤 3-6。
4. 丽春红品红染色液滴染 5-7min，蒸馏水洗 2 次，每次 10-15s。
5. 磷钼酸溶液分化处理 2-3min。
6. 倾去上液，切片不用水洗，用弱酸工作液将苯胺蓝染色液稀释 3-4 倍染色 1-3min。（*见注意事项 5*）
7. 用弱酸工作液洗去苯胺蓝溶液后，继续滴加弱酸工作液覆盖切片处理 2min。
8. 95%乙醇快速脱水 3-5s。 无水乙醇脱水 2 次，每次 5-10s。 （*见注意事项 6*）
9. 二甲苯透明 2 次，每次 1-2min。 中性树胶封固。

染色结果：

胶原纤维	蓝色
肌纤维、胞质、纤维素、角蛋白和红细胞	不同程度的红色
胞核	蓝褐色

注意事项：

1. 切片脱蜡应尽量干净。
2. 本品媒染液和酸性分化液均为优化配方，均为不含危险化学品的无色溶剂，与经典含苦味酸的 bouin 媒染剂和酸性乙醇分化液性状有较大区别，属于正常现象。以上两种试剂均为原试剂优化替代品，如您更倾向于经典配方可自行替换对应步骤所用试剂。
3. 酸性分化液的分化时间应该依据切片薄厚，组织的类别和新旧而定。
4. 磷钼酸溶液的作用一方面是使染上红色的胶原纤维被分化成无色或淡红色，而肌纤维纤维素等仍呈鲜红色；另一方面对胶原纤维又起媒染作用，使胶原纤维与大分子染料的苯胺蓝染液较易结合。
5. 冰冻切片相对于石蜡切片来说，胶原纤维容易出现苯胺蓝过染情况，所以建议用弱酸溶液稀释苯胺蓝染色液 3-4 倍再进行染色，对于皮肤等易染组织，可以再次缩短染色时间。
6. 若组织经过 95%乙醇和无水乙醇处理后出现红色或蓝色脱色情况，在用弱酸工作液清洗组织后，可以甩干多余液体，直接晾干，然后进行后续二甲苯透明和封片步骤。
7. 弱酸溶液可使色彩更清晰鲜艳，如使用量大可购买 G2940-弱酸水溶液进行替代。
8. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

参考文献：

[1] Dan Xiao,Yue Zhang,Rui Wang,et al. Emodin alleviates cardiac fibrosis by suppressing activation of cardiac fibroblasts via upregulating metastasis associated protein 3. Acta Pharmaceutica Sinica B. April 2019. (IF 5.808)
[2] Na Li,Lin Zhou,Weilong Xie,et al. Alkaline phosphatase enzyme-induced biomineralization of chitosan scaffolds with enhanced osteogenesis for bone tissue engineering. Chemical Engineering Journal. September 2019;371:618-630. (IF 8.355)
[3] Ke Xue,Jun Zhang,Cong Li,et al. The role and mechanism of transforming growth factor beta 3 in human myocardial infarction-induced myocardial fibrosis. Journal of Cellular and Molecular Medicine. April 2019. (IF 4.658)

V02

Modified Masson's Trichrome Stain Kit

Cat: G1346
Size: 8×50mL/8×100mL
Storage: 2-8℃, avoid light, valid for 1 year.

Kit Components

Reagent	8×50mL	8×100mL	Storage
Reagent(A): Mordant Solution(3×)	50mL	100mL	RT, avoid light
Reagent(B): Celestite Blue Solution	50mL	100mL	2-8℃, avoid light
Reagent(C): Mayer Hematoxylin Solution	50mL	100mL	2-8℃, avoid light
Reagent(D): Acid Differentiation	50mL	100mL	RT
Reagent(E): Ponceau-Acid Fuchsin Solution	50mL	100mL	RT, avoid light
Reagent(F): Phosphmolybic Acid Solution	50mL	100mL	RT, avoid light
Reagent(G): Aniline Blue Solution	50mL	100mL	RT, avoid light
Reagent(H): Weak Acid Solution(3×)	50mL	100mL	RT

Introduction

Narrowly speaking, connective tissue refers to the three types of fibers it contains: collagen fibers, reticular fibers, and elastic fibers. Collagen fibers are the most widely distributed and abundant type of fiber. Masson trichrome staining, also known as Masson staining, is the most classic method in connective tissue staining and an authoritative and classic technical method for collagen fiber staining. The so-called trichrome staining usually refers to staining the cell nucleus and selectively displaying collagen and muscle fibers. The dyeing principle of this method is related to the size of anionic dye molecules and the permeability of tissues: the size of molecules is reflected by their molecular weight. Small molecular weights are easy to penetrate tissues with dense structures and low permeability, while large molecular weights can only enter tissues with loose structures and high permeability. However, the molecular weight of light green or aniline blue is large, so after Masson staining, muscle fibers appear red, while collagen fibers appear green or blue, mainly used to distinguish between collagen fibers and muscle fibers.

The difference between the Modified Masson's Trichrome Stain Kit and the conventional Masson Trichrome Stain Kit is that the nucleus of the cell is light stained with Celestite Blue Hematoxylin Solution. It has the following characteristics: short differentiation time,clear and bright colors; wide application range, suitable for paraffin section, frozen section and other staining of tissues; long preservation time of stained sections and not easy to fade. Modified Masson's Trichrome Stain Kit shows that collagen fiber is blue, muscle fiber, cytoplasm, cellulose, keratin and erythrocytes are red, and nucleus is blue, which was mainly used to distinguish collagen fiber and muscle fiber.

Self Provided Materials

G2161-Neutral Buffered Formalin Fixative, 10% or P1110-Paraformaldehyde,4%, Bouin fixative, distilled water, series ethanol, xylene, or G2150 environmental protection tissue transparent dewaxing solution, dye tank

Protocol(*for reference only*)

Before staining, prepare the working solutions according to the required amount as follows:Mix Reagent (A): Mordant Solution (3×) with distilled water at a ratio of 1:2 to obtain Mordant Solution (1×).Mix Reagent (H): Weak Acid Solution (3×) with distilled water at a ratio of 1:2 to obtain Weak Acid Working Solution.

For paraffin sections

1. Fix tissues in 10% neutral formalin or Paraformaldehyde,4% , then dehydrate and embed.
2. Cut the section in 3-6μm thick, dewax to distilled water.
3. Incubate the section in Mordant Solution(1×) for 8-12h at RT or incubate the section in 57-60℃ incubator for 1h, then wash with distilled water for three times, each for 3min. (*See Note 2*)
4. Drop Celestite Blue Solution onto the section and stain for 2-3min. Slightly wash with distilled water twice, each for 10-15s.
5. Drop Mayer Hematoxylin Solution onto the section and stain for 2-3min. Slightly wash with distilled water twice,each for 10-15s.
6. Differentiate by Acid Differentiation for several seconds and wash with distilled water to stop differentiation.

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Rinse in running water for 10min(Return to Blue). (*See Note 2*)
7. Drop Ponceau-Acid Fuchsin Solution onto the section and stain for 5-10min. Slightly wash with distilled water twice,each for 10-15s .
8. Treat with Phosphmolybic Acid Solution for 5-10min.
9. Discard the remaining dye solution without washing and stain with Aniline Blue Solution for 3-5min.
10. Wash the section with Weak Acid Working Solution, and rinse in Weak Acid Working Solution for 2mins.
11. Dehydrate quickly in 95% ethanol. Dehydrate in absolute ethanol twice for each time 5-10s.(*See Note 6*)
12. Transparent by xylene twice for each time 1-2mins. Seal with resinene.

For frozen sections

1. Fix tissues in 10% neutral formalin or Paraformaldehyde,4%, dehydrate by gradient sucrose and embed in OCT to prepare frozen sections.
2. Cut the section in 8-15μm thick. Before staining, place the slices in distilled water and restore to room temperature for 3min.
3. The staining steps are the same as steps 3-6 for paraffin sections.
4. Drop Ponceau-Acid Fuchsin Solution onto the section and stain for 5-7min. Slightly wash with distilled water twice,each for 10-15s .
5. Treat with Phosphmolybic Acid Solution for 2-3min.
6. Discard the remaining dye solution without washing and stain with Aniline Blue Solution(diluted 3-4 times by Weak Acid Working Solution)for 1-3min. (*See Note 5*)
7. Wash the section with Weak Acid Working Solution, and rinse in Weak Acid Working Solution for 2mins.
8. Dehydrate quickly in 95% ethanol. Dehydrate in absolute ethanol twice for each time 5-10s.(*See Note 6*)
9. Transparent by xylene twice for each time 1-2mins. Seal with resinene.

Result

Collagen Fiber	Blue
Muscle Fiber, Cytoplasm, Cellulose, Keratin and Erythrocytes	Red
Nucleus	Bluish Brown

Note

1. Section dewaxing should be as clean as possible.
2. The Mordant Solution and Acid Differentiation of this kit are optimized formulas, which are colorless solvents without dangerous chemicals. They are quite different from the properties of classical picric acid mordant and acid ethanol differentiation solution, witch belongs to normal phenomena. The above two reagents are optimized substitutes for the original reagent. If you prefer the classic formula, you can replace the reagents used in the corresponding steps by yourself.
3. The differentiation time of Acid Differentiation should be determined by the thickness of section, the type of tissue and the old and new.
4. Phosphmolybic Acid Solution has two effects. One is that Phosphmolybic Acid Solution can make the collagen fiber dyed red differentiate into colorless or light red, while the muscle fiber cellulose is still bright red; the other one is that it can also play a role of mordant dyeing on the collagen fiber, so that the collagen fiber is easy to combine with Aniline Blue Solution of macromolecular dye.
5. Compared to paraffin sections, frozen sections are prone to staining collagen fibers with aniline blue. Therefore, it is recommended to dilute the Aniline Blue Solution with a Weak Acid solution 3-4 times before staining. For skin and other easily stained tissues, the staining time can be shortened again.
6. If the tissue shows red or blue decolorization after treatment with 95% ethanol and absolute ethanol, after cleaning the tissue with the Weak Acid Working Solution, you can spin off the excess liquid, let it air-dry directly, and then proceed with the subsequent xylene clearing and mounting steps.
7. Weak Acid Solution can make the color more clear and bright. If the use amount is large, you can buy G2940-Weak Acid Solution instead.
8. For your safety and health, please wear experimental clothes and disposable gloves.