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	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	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：

10%的福尔马林、蒸馏水、系列乙醇、二甲苯、染缸

操作步骤： *（仅供参考）*

1. 组织固定于 10%的福尔马林中，常规脱水包埋。
2. 切片厚 4μm，常规脱蜡至水。
3. 切片入媒染液浸染，于室温作用一晚或置入 57℃-60℃的温箱内 1h 进行媒染，然后流水冲洗 10min。（见注意事项 2）
4. 天青石蓝染色液滴染 2~3min，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，水洗终止分化，蒸馏水冲洗 10min。（见注意事项 2）
7. 丽春红品红染色液滴染 10min，蒸馏水洗 2 次，每次 10-15s。
8. 磷钼酸溶液处理约 10min。
9. 倾去上液，切片不用水洗，直接滴加苯胺蓝染色液染 5min。

10. 用弱酸溶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。
11. 95%的乙醇脱水 30s。 无水乙醇脱水 2 次，第一次 30s，第二次 1min。
12. 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，与经典苦味酸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液较易结合。
5. 苯胺蓝液染色后用弱酸溶液处理，目的是除去原浆内的蓝色，使染色鲜艳和清晰。若 Zenker 液固定的组织，弱酸溶液处理可延长至 5min。
6. 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。
7. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

相关文献:

[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)

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	50mL	100mL	RT
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 Solution	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): Acetic Acid Solution	50mL	100mL	RT

Introduction

Masson Trichrome Stain 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.

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

10% Formalin, Distilled water, Series of ethanol, xylene, Dye vat.

Protocol(*for reference only*)

1. Fix tissues in 10% formalin, then dehydrate and embed.
2. Cut the section in 4μm thick, dewax to distilled water.
3. Incubate the section into Mordant Solution in 37℃ overnight or incubate in 57-60℃ incubator for 1h, then wash with running water for 10mins. （See note 2）
4. Drop Celestite Blue Solution onto the section and stain for 2-3mins.Slightly wash with distilled water twice,each for 15-30s.
5. Drop Mayer Hematoxylin Solution onto the section and stain for 2-3mins. Slightly wash with distilled water twice,each for 15-30s.
6. Differentiate by Acid Differentiation Solution for several second.Rinse in running water for 10mins. （See note 2）

7. Drop Ponceau-Acid Fuchsin Solution onto the section and stain for 10mins. Slightly wash with distilled water twice,each for 15-30s .
8. Treat with Phosphmolybic Acid Solution for 10mins.
9. Discard the remaining dye solution and directly stain with Aniline Blue Solution for 5mins without washing with water.
10. As the radio of 1:2, mix the Acetic Acid Solution and water to prepare Acetic Acid working Solution, and rinse in Acetic Acid working Solution for 2mins.
11. Dehydrate quickly in 95% ethanol. Dehydrate in absolute ethanol 3 times for each time 5-10s.
12. Transparent by xylene three times 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 Solution of this product 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, and belong 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 Solution 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. Treat the section with Acetic Acid Solution after Aniline Blue Solution staining to remove the blue in the original pulp and make the dyeing bright and clear. If the tissue is fixed with Zenker solution, the treatment with Acetic Acid Solution can be prolonged to 5 mins.
6. Acetic Acid Solution can make the color more clear and bright. If the use amount is large, can replace it with 0.1-0.3% acetic acid solution by yourself .
7. For your safety and health, please wear experimental clothes and disposable gloves.

Reference

[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)

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