未脱钙骨硬组织切片 Masson 染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
烤箱	天津市莱玻瑞仪器设备有限公司	GFL-230
载玻片	Servicebio	
正置光学显微镜	日本尼康	Nikon Eclipse E100
成像系统	日本尼康	Nikon DS-U3

2、 主要实验试剂

试剂名称	厂家	货号
无水乙醇	国药集团化学试剂有限公司	100092683
二甲苯	国药集团化学试剂有限公司	10023418
Masson 染液套装	Servicebio	G1006
中性树胶	国药集团化学试剂有限公司	10004160
乙二醇乙醚乙酸酯	麦克林	E808814-2.5L

二、实验步骤

1、切片脱塑至水：依次将切片乙二醇乙醚乙酸酯I 6 h，37℃，乙二醇乙醚乙酸酯II过夜，37℃，乙二醇乙醚乙酸酯III室温 10-15 min，乙二醇乙醚乙酸酯IV室温 10-15 min，100%I乙醇 10 min，100%II乙醇 10 min，95%乙醇 10 min，90%乙醇 10 min，80%乙醇 10 min，自来水洗。

2、切片浸入Masson A液中浸泡过夜（约15个小时）。

3、切片浸泡于 Masson A 液内于 37℃烤箱内加热 30 min；同时将 Masson D 液及 Masson F 液放于 37℃烤箱内预热。

4、自来水洗 30 S，组织黄色不明显即可。

5、临用前将Masson B液及Masson C液两液等量混合，切片入混合液浸染1分钟，流水稍洗。

6、1%盐酸酒精分化1 min左右，核呈灰黑色，背景几乎无色或淡灰色。

7、自来水稍洗，稍稍沥干多余水分，Masson D液浸染6分钟，整个组织呈现亮红色，如果红色过浅可适当延长染色时间。

8、将多余水分尽量沥干,但是时间不要太长,组织不能干片，Masson E液处理约1分钟；

9、不水洗，稍微沥干多余 Masson E 液直接入 Masson F 液染色 2s-10 s。

10、三缸 1%冰醋酸漂洗分化，每缸大约 5 s 左右；三缸无水乙醇脱水，第一缸 5 s，第二，三缸 10 s,入第一缸正丁醇 30 s，第二缸 2 min，两缸二甲苯透明，各 5 min， 中性树胶封片。

三、结果判读：

胶原纤维呈蓝色；肌纤维、纤维素和红细胞呈红色。

四、注意事项：

1、Masson A 液染完后水洗要稍快，切片洗干净即可；

2、根据切片的数量对混合的染液进行更换，也可以现配现用；

3、Masson D 液的染色程度要控制好，胶原部分不能是红色或者太红，会影响后续 Masson F 液的着色；

4、冰醋酸分化过程中，若是分化过度，胶原蓝色太浅，若分化不足，易与红色叠加呈紫蓝色。

Undecalcified Bone Masson Stain Report

1. Experimental equipment and reagents

1.1 Equipment

Brand name	Manufactures	Model
Oven	Tianjin Labotery instrument equipment Co. Ltd	GFL-230
Microslide	Servicebio	
Optical Microscopy	NIKON	Nikon Eclipse E100
Imaging system	NIKON	Nikon DS-U3

1.2 Reagent

Reagent	Manufactures	Cat
Ethanol absolute	Sinopharm Chemical Reagent Co.,Ltd	100092683
Xylenes	Sinopharm Chemical Reagent Co.,Ltd	100023418
Masson staining solution	Servicebio	G1006
Neutral balsam	Sinopharm Chemical Reagent Co.,Ltd	10004160
Ethylene glycol ethyl ether acetate	Macklin	E808814-2.5L

2. Experiment procedure

2.1 Remove the embedding agent and rehydration

Put tissue section into ethylene glycol ethyl ether acetate I 6 h at 37 ℃ and ethylene ether acetate II overnight at 37 ℃. Then put them into ethylene glycol ethyl ether acetate III 10-15 minutes at room temperature and ethylene glycol ethyl ether acetateIV 10-15 minutes at room temperature. The sections were rehydrated in 100%I - 100%II - 95% - 90% - 80% alcohol. Each step takes anywhere for 10 minutes. Finally rinse with running water.

2.2 The slices ware immersed in Masson staining solution A overnight (about 15 hours).

2.3 The slices were immersed in Masson staining solution A and heated in an oven at 37℃ for 30 minutes. Meanwhile, preheat the Masson staining solution D and Masson staining solution F in

37℃ oven.

2.4 Wash with running water for 30 s until the yellow tissue is not obvious.

2.5 Mix Masson staining solution B and Masson staining solution C in equal amount, and stain section 1 minute, then wash it with running water.

2.6 The nuclei were grayish black after differentiated by 1% hydrochloric acid alcohol for about 1 minute**.** The background was almost colorless or light gray.

2.7 The running water was slightly washed and the excess water was slightly drained. The whole tissue was bright red after Masson staining solution D was impregnated for 6 minutes. If the coloring is too light, the dyeing time can be extended appropriately.

2.8 Drain the excess water as long as possible, but do not take too long time to dry the tissue. Finally, Masson staining solution E treatment for about 1 minute.

2.9 Slightly drain excess Masson staining solution E and dye it directly into Masson staining solution E for 2-10 s.

2.10 Put sections into three cylinders of 1% acetic acid rinsing and differentiation for 5 minutes. Three cylinders of anhydrous ethanol were used for dehydration and the side time is 5 s and 10 s respectively. Then three cylinders of xylenes were used for transparency for 5 minutes. Finally, sealed the sections with neutral balsam.

3. Result determination

Collagen fibers are blue. Muscle fibers, cellulose and red blood cells are red.

4. Matter need attention

4.1 The water should be washed slightly faster after Masson staining solution A is dyed and the sections can be washed clean.

4.2 The staining degree of Masson staining solution D should be controlled. The collagen part should not be red or deep crimson color, which will affect the staining of subsequent Masson staining solution F.

4.3 In the process of glacial acetic acid differentiation, if the differentiation is excessive, the blue color of collagen is too light. On the contrary, it is easy to superposition with red color into purple blue.