黑色素染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
脱水机	DIAPATH	Donatello
包埋机	武汉俊杰电子有限公司	JB-P5
病理切片机	上海徕卡仪器有限公司	RM2016
冻台	武汉俊杰电子有限公司	JB-L5
组织摊片机	浙江省金华市科迪仪器设备有限公司	KD-P
烤箱	天津市莱玻瑞仪器设备有限公司	GFL-230
载玻片	Wanwu	
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
黑色素染液套装	Wanwu	G1067
中性树胶	国药集团化学试剂有限公司	10004160
氨水	国药集团化学试剂有限公司	10002118

二、实验步骤

黑色素工作液配制：取黑色素染液 A10ml，逐滴加入浓氨水至产生沉淀后，继续滴加氨水，边滴边摇动，使产生的沉淀又复溶解，溶液变清。再滴入黑色素染液 A 数滴使溶液呈轻微混浊，最后加入 20ml 的蒸馏水，过滤后即可使用。

1、石蜡切片脱蜡至水：依次将切片放入二甲苯I20min-二甲苯II20min-无水乙醇I5min-无水乙醇II5min-75%酒精 5min，自来水洗，蒸馏水浸洗 3-5 遍。

2、工作液孵育：切片浸入黑色素工作液并加盖，置于 4℃避光反应 12-18h，蒸馏水洗 3 遍。

3、复染 VG：黑色素染液 B 9ml 加入黑色素染液 C 1ml（配制后可反复使用多次）混合成 VG 染液（按比例配制用多少配多少），染 1min，快速水洗，无水乙醇三缸快速脱水 3s、5s、30s。

4、透明封片：两缸干净的二甲苯透明各 20s、5min（二甲苯专用不与其他二甲苯共用），中性树胶湿封；

5、显微镜镜检，图像采集分析。

三、结果判读：

黑色素、亲银细胞胞质呈黑色，胶原纤维呈红色，肌纤维和红细胞呈黄色。

四、注意事项：

1、配工作液和孵育时用的容器，在用之前必须用蒸馏水清洗干净，否则易出现杂质和沉淀。

Masson-Fontana staining experimental report

1. Lab equipment and reagents

A. Lab equipment

Items	Manufacturer	Model
Dehydrator	DIAPATH	Donatello
embedding machine	Wuhan Junjie Electronics Co., Ltd.	JB-P5
Pathology microtome	Shanghai Leica Instruments Co., Ltd.	RM2016
Frozen platform	Wuhan Junjie Electronics Co., Ltd.	JB-L5
Water Bath-Slide Drier	Zhejiang Jinhua Kedi Instrumental Equipment CO.,LTD	KD-P
Laboratory oven	Tianjin Labotery Instrument Equipment Co., Ltd.	GFL-230
Microscope slide	Wanwu	
Upright optical microscope	Nikon Japan	Nikon Eclipse E100
Imaging system	Nikon Japan	NIKON DS-U3

B. Chemical Reagents

Items	Manufacturer	Model
Absolute alcohol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Fontana Masson Stain Kit	Wanwu	G1067
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160
Ammonium hydroxide	Sinopharm Chemical Reagent Co., Ltd.	10002118

2. Experimental steps

Preparation of Masson-Fontana staining solution: take Masson-Fontana staining solution A for 10ml, After adding concentrated ammonium hydroxide drop by drop until precipitation occurs, continue to drip Ammonium hydroxide drop by drop, and shake while dripping, so that the resulting precipitate redissolves and the solution turns clear. Then add a few drops of Masson-Fontana staining solution A to make the solution slightly turbid, finally add 20ml of distilled water and use it after filtering.

1.Paraffin section deparaffinize and rehydrate: put the slides into xylene I 20minutes-xylene II 20 minutes-absolute ethanol I 5 min-absolute ethanol II 5 min-75% alcohol for 5 min, then tap water washing and with distilled water washing for 3-5 times .

2.Working solution incubation: put the slides in the Masson-Fontana solution and covered, place at 4°C and avoiding light for 12-18h, and distilled water washing for three times.

3.VG Counterstaining: add Masson-Fontana solution B 9ml into Masson-Fontana solution C 1ml (can be used repeatedly for many times after preparation) and mix it into VG staining solution (proportionally prepared according to the ratio), stain for 1min, wash quickly, absolute alcohol rapid dehydration for 3 times , 3s, 5s, 30s each time.

4.Clearing and sealing: two cylinders of clean xylene transparent for 20s, 5min each time (xylene is not shared with other xylene), neutral balsam sealing.

5. Microscope examination, images collection and analysis.

3. Results

The cytoplasm of melanin and silver-philic cell is black, collagen fiber is red, and muscle fibers and red blood cell are yellow.

4. Note

The working solution and the container used for incubation should be cleaned with distilled water before usage, or impurities and precipitation will be occurred.