荧光桃红 B 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	G6004
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
荧光桃红 B	阿拉丁	G1725052
氯化汞	国药集团化学试剂有限公司	10013616
甲醛	国药集团化学试剂有限公司	100100620
氯化钙	国药集团化学试剂有限公司	10005860
苏木素染液	Wanwu	G1004
分化液	Wanwu	G1005-3
返蓝液	Wanwu	G1005-4
中性树胶	国药集团化学试剂有限公司	10004160

二、试剂配制

固定液：氯化汞 8g+蒸馏水 100ml　取上清液：甲醛=9：1 混匀即可

荧光桃红 B 染色液：0.5g 荧光桃红 B 溶于 100ml 0.1%CaCl2 溶液中

三、实验步骤

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。

2、苏木素染色：切片入苏木素染色 1-3min，水洗，分化液分化，水洗，返蓝液返蓝，水洗，蒸馏水洗。

3、荧光桃红 B 染色：荧光桃红 B 染液液滴染 5-15min，蒸馏水洗去多余的红色背景，在显微镜下控制。

4、脱水封片：无水乙醇3缸快速脱水，二甲苯透明5min，中性树胶封片。

5、显微镜镜检，图像采集分析。

四、结果判读：

潘氏细胞内的嗜酸性颗粒呈红色，细胞核呈浅蓝色，背景淡粉或者近无色。

五、注意事项：

1、组织取材后要立即用染液套装中的染固定液进行固定，固定24h以上。

2、细胞核染色不可染深，浅染至核结构清晰即可。

3、显微镜下用水分化背景时，严格控制分化程度，使潘氏细胞和细胞核的对比明显。

Phloxine B 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	G6004
Upright optical microscope	Nikon Japan	Nikon Eclipse E100
Imaging system	Nikon Japan	NIKON DS-U3

B. Chemical Reagents

Item	Manufacturer	Model
Absolute alcohol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Phloxine B	Aladdin	G1725052
Mercury chloride	Sinopharm Chemical Reagent Co., Ltd.	10013616
Formaldehyde	Sinopharm Chemical Reagent Co., Ltd.	100100620
Calcium chloride	Sinopharm Chemical Reagent Co., Ltd.	10005860
Hematoxylin staining solution	Wanwu	G1004
Differentiation solution	Wanwu	G1005-3
返蓝液	Wanwu	G1005-4
Rhamsan gum	Sinopharm Chemical Reagent Co., Ltd.	10004160

2.Reagent preparation

Fixative: mercury chloride 8g + 100ml distilled water , take the supernatant: formaldehyde = 9: 1 and mix them.

Phloxine B staining solution: taking 0.5g Phloxine B dissolve in 100ml 0.1% CaCl2 solution

3.Experimental steps

(1)Paraffin section deparaffinization and rehydration: 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.

(2)Hematoxylin staining: put slides into hematoxylin solution staining for 1-3min, water washing, differentiate with differentiation solution, water washing, return to blue solution, water washing, and distilled water washing.

(3)Phloxine B staining: staining with phloxine B solution for 5-15 minutes, wash away the excess red background with distilled water, control under a microscope.

(4)Dehydration and sealing: dehydrate with absolute ethanol in 3 cylinders, transparent with xylene for 5min, and xylene for 5min, rhamsan gum sealing.

(5)Microscope examination, images collection and analysis.

4.Results

The eosinophilic granules in Paneth cell are red, the nucleus is light blue, and the background is light pink or nearly colorless.

5.Note

1. After taken out the tissue from the fixative solution, it should be fixed immediately with the fixing solution in the staining solution kit and fix for more than 24h.

2. Cell nucleus staining should not be stained deeply, and it can be stained lightly until the nuclear structure is clear.

3. When differentiating the background under a microscope, should be strictly control the degree of differentiation so that the confrontation between Paneth cells and the nucleus is obvious.