间苯二酚碱性品红染色实验报告

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
间苯二酚碱性品红染液套装	Wanwu	G1054
中性树胶	国药集团化学试剂有限公司	10004160

二、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、间苯二酚碱性品红染色：切片入间苯二酚品红染液 A 中浸染并加盖，室温下 4h，取出切片流水冲洗，至玻片流水呈无色即可。

3、背景分化：切片入 1%盐酸酒精分化液快速分化 2s，自来水洗 5s，镜检，可反复快速分化、水洗并镜检直至观察到清晰的紫色的弹力纤维，背景淡紫色至几乎无色即可；

4、复染 VG：间苯二酚碱性品红染液 B 9ml 加入间苯二酚碱性品红染液 C 1ml 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，依次 3s、5s、30s。

5、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。

6、显微镜镜检，图像采集分析。

三、染色判读：

弹力纤维紫红色，胶原纤维红色，背景其他成分黄色。

四、注意事项：

1、VG复染过程中水洗要快，防止红色褪掉，无水乙醇也要快洗，防止黄色变暗。

Resorcin-Fuchsin 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
Resorcin-Fuchsin solution kit	Wanwu	G1054
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160
Nuclear fast red	Wanwu	G1035
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

2. 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) Resorcin-Fuchsin staining: dip the slides into resorcin-fuchsin staining solution A and coved,

place at room temperature for 4h, take out the slides rinse with running water until water in the slide is colorless.

(3) Background differentiation: put the slides into 1% hydrochloric acid alcohol differentiation solution for rapid differentiation for 2s, tap water washing for 5s, microscope examination, can be rapidly differentiated, washed and microscopically examination until clear purple elastic fibers are observed, and the background is light purple to almost colorless;

(4) Counterstaining VG: add resorcin-fuchsin staining solution B 9ml into resorcinol basic magenta staining solution C 1ml and mix into VG staining solution (according to the ratio), stain for 1min, water washing quickly, and quickly dehydrate in three-cylinder of absolute ethanol, 3s, 5s, 30s respectively.

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

(6) Microscope examination, image collection and analysis.

3. Staining results:

Elastic fiber are purple red, collagen fiber are red, and other ingredients in the background are yellow.

4. Attention:

1. Wash quickly during the VG counterstaining process to prevent the red from fading, same as anhydrous ethanol washing to prevent the yellow from darkening.