红氨酸铜染色实验报告

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
红氨酸	Sigma	379387
醋酸钠	国药集团化学试剂有限公司	10018818
核固红	Wanwu	G1035
中性树胶	国药集团化学试剂有限公司	10004160

二、试剂配制

0.1%的红氨酸乙醇液：红氨酸 10mg+无水乙醇 10ml

10%的醋酸钠：醋酸钠 10g+超纯水 100ml

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，蒸馏水洗 3 次。

2、红氨酸铜染色：将0.1%的红氨酸乙醇液：10%的醋酸钠以1：20比例混匀，切片浸入混合液中加盖于37℃水浴锅处理约3天。切片入70%的乙醇浸洗2次，每次10min，入无水乙醇浸洗2次，每次3h；

3、染核：蒸馏水浸洗3次，将乙醇洗干净，复染核固红3min，自来水水洗；

4、脱水透明：无水乙醇3缸脱水，正丁醇透明5min，二甲苯透明5min，中性树胶封片。

5、显微镜镜检，图像采集分析。

三、结果判读：

铜盐沉积处呈深绿黑色，细胞核呈红色。

四、注意事项：

1、0.1%的红氨酸乙醇液常温避光保存；

2、红氨酸铜混合液要现配现用，用完弃掉。

Copper red acid 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
Dithio oxamide	Sigma	379387
Acetic acid	Sinopharm Chemical Reagent Co., Ltd.	10018818
Nuclear fast red	Wanwu	G1035
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

2. Reagent preparation

0.1% Dithio oxamide ethanol solution: Dithio oxamide10mg + absolute ethanol 10ml

10% acetic acid: acetic acid 10g + ultra-pure water 100ml

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 and distilled water washing for 3 times.
(2) Copper red acid staining: 0.1% Dithio oxamide ethanol solution: 10% sodium acetate mix at a ratio of 1:20, and put the slides into the mixed solution and covered in a 37°C water bath for about 3 days. Put the slides into 70% ethanol twice for 10 minutes each time, and absolute ethanol washing for twice, 3 hours each time;
(3) Nuclear staining: distilled water washing for 3 times, wash out ethanol, nuclear fast red counterstaining for 3min, then tap water washing;
(4) Dehydration and sealing: dehydrate with absolute ethanol in 3 cylinders, transparent with n-butanol for 5min, and xylene for 5min, neutral balsam sealing.
(5) Microscope examination, image collection and analysis.

4. Results

The copper salt deposition are dark green and black, and the nucleus is red.

5. Note

1. 0.1% Dithio oxamide ethanol solution should be stored at room temperature(lightproof);

2. The copper red acid mixed solution should be ready for use and discard after usage.