Grimelins 硝酸银染色实验报告

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	天津市莱玻特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
硝酸银	国药集团化学试剂有限公司	10018461
冰醋酸	国药集团化学试剂有限公司	10000218
醋酸钠	国药集团化学试剂有限公司	10018718
对苯二酚	国药集团化学试剂有限公司	10011317
无水亚硫酸钠	国药集团化学试剂有限公司	S112302
中性树胶	国药集团化学试剂有限公司	10004160

二、试剂配制

1%硝酸银溶液： 硝酸银 1 g+超纯水 100mL

醋酸盐缓冲液(母液)： A 液：1.21ml 冰醋酸+98.7ml 超纯水

B 液：2.72g 醋酸钠+100ml 超纯水

还原液：对苯二酚 1g+无水亚硫酸钠 2.5g+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I 20min-二甲苯II 20min-无水乙醇I 5min-无水乙醇II 5min-75%酒精 5min，自来水洗，蒸馏水 3-5 次。

2、配制银液孵育液：将A液：B液=1:9混合成醋酸盐缓冲液，取1%的硝酸银3ml+醋酸盐缓冲液10ml+超纯水87ml

3、硝酸银染色：将银液孵育液置于60℃预热，切片浸入银液60℃孵育3h。

4、还原染色：从银液中取出切片，蒸馏水急速浸洗1次，切片入45℃已预热好的还原液中1h，取出切片，蒸馏水浸洗数次；

5、脱水透明：切片依次入无水乙醇I 5min -无水乙醇II 5min-无水乙醇III5min -二甲苯I5min -二甲苯II5min透明，中性树胶封片。

6、显微镜镜检，图像采集分析。

三、结果判读：

嗜银细胞主要呈花瓶形、梭形、蝌蚪形、圆形和羽毛球拍等形状。细胞核不着色，细胞质被染成棕褐色，胞浆内嗜银颗粒丰富呈棕黑色。

四、注意事项：

1、混合液和银液孵育液都要现混现用，混合液的母液可长期保存。

2、在染色过程中，蒸馏水急速浸洗需要快速操作。

Grimelins Silver Nitrate Staining Experiment Report

I. Experimental Equipment and Reagents

1. Experimental equipment

Equipment name	Manufacturer	Model No.
Dehydrator	DIAPATH	Donatello
Embedding center	Wuhan Junjie Electronics Co., Ltd.	JB-P5
Pathological microtome	Shanghai Leica Instrument Co., Ltd.	RM2016
Cooling plate	Wuhan Junjie Electronics Co., Ltd.	JB-L5
Tissue spreading water bath	Zhejiang Jinhua Kedi Instrumental Equipment Co., Ltd.	KD-P
Oven	Tianjin Leibo Terry Equipment Co., Ltd.	GFL-230
Microscope slide	Servicebio	
Upright electron microscope	JAPAN NIKON	NIKON ECLIPSE E100
Imaging system	JAPAN NIKON	NIKON DS-U3

2. Main experiment reagents

Reagent name	Manufacturer	Item No.
Anhydrous ethanol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Silver nitrate	Sinopharm Chemical Reagent Co., Ltd.	10018461
Glacial acetic acid	Sinopharm Chemical Reagent Co., Ltd.	10000218
Sodium acetate	Sinopharm Chemical Reagent Co., Ltd.	10018718
Hydroquinone	Sinopharm Chemical Reagent Co., Ltd.	10011317
Anhydrous Sodium Sulfite	Sinopharm Chemical Reagent Co., Ltd.	S112302
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

II. Dyeing Solution Preparation

1% silver nitrate solution: 1g Silver nitrate + 100mL ultrapure water

Acetate buffer (mother liquid) : Solution A: 1.21ml glacial acetic acid + 98.7ml ultrapure water

Solution B: 2.72g sodium acetate + 100ml ultrapure water

Reducing solution: 1g Hydroquinone + 2.5g anhydrous sodium sulfite + 100ml ultra-pure water

III. Experiment Steps

1. Deparaffinize the paraffin sections to water: Place the sections in order in Xylene I for 20min - Xylene II for 20min - Anhydrous ethanol I for 5min - Anhydrous ethanol II for 5min - 75% Ethyl alcohol for 5min , wash with tap water, and 3-5 times with distilled water.

2. Prepare silver solution incubation medium: Mix solution A: solution B=1:9 into acetate buffer, take 3ml 1% silver nitrate + 10ml acetate buffer + 87ml ultrapure water.

3. Silver nitrate staining: Place the silver solution incubation medium at 60 ℃ for preheating, and immerse the sections in the silver solution for incubation at 60 ℃ for 3 hours.

4. Reducing dyeing: Take out the sections from the silver solution, quickly dip it in distilled water for 1 time, then put the sections into the 45℃ preheated reducing solution for 1 hour, take out the sections, dip and wash in distilled water for several times.

5. Dehydration and transparency: Put the sections in order into Anhydrous ethanol I for 5min - Anhydrous ethanol II for 5min - Anhydrous ethanol III for 5min - Xylene I for 5min - Xylene II for 5min for transparency, at last, seal with neutral balsam.

6. Microscope inspection, image acquisition and analysis.

IV. Interpretation of Results:

The argyrophil cells are mainly in the shape of vases, spindle, tadpole, circle and badminton racket. The nucleus is non-staining, the intracytoplasmicis argyrophilic grain are plentiful and in brown-black color.

V. Precautions:

1. Both the mixed solution and the silver solution incubation medium should be mixed freshly and use immediately, the mother liquid of the mixed solution can be stored for a long time.

2. During the dyeing process, rapid dip and wash in distilled water requires quick operation.