

## 甘氨酸银浸镀神经染色

### 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	上海徠卡仪器有限公司	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
甘氨酸银染液套装	Wanwu	G1052
中性树脂	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，蒸馏水洗多遍，洗干净。

2、甘氨酸银染液 C 染色：切片入甘氨酸银染液 C5min，蒸馏水洗 3 次。

3、甘氨酸银染液 B 处理：切片入甘氨酸银染液 B 中处理 3-5min。

4、甘氨酸银染液 A 染色：将切片取出，迅速甩掉组织上残余的甘氨酸银染液 B，放入甘氨酸银染液 AI(已提前 45°C预热好)中，时刻观察还原效果，几秒后取出切片并迅速将切片入甘氨酸银染液 AII(已提前 45°C预热好)中，几秒后快速拿出，蒸馏水洗。

若切片背景较深则可以用甘氨酸银染液 D 快速处理 1s，并用蒸馏水洗 3 次。

**5、脱水封片:**切片依次放入无水乙醇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:**

神经轴突、神经细胞内原纤维、神经原纤维缠结、老年斑及树突呈黑色, 背景金黄色。

### **四、注意事项:**

- 1、在甘氨酸银液处理前后都必须用蒸馏水清洗干净, 否则易出现杂质。
- 2、每次只能做一张切片, 便于控制效果, 且还原过程一定要迅速, 没控制好片子容易脏。
- 3、所有的染液都需要避光保存, 甘氨酸银液同一天内可使用 3-5 次, 还原液每次要换新。

## Glycine Silver Immersion Plating Nerve Staining

### 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	Wanwu	
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
Glycine silver staining solution kit	Wanwu	G1052
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

### II. Experimental procedure

**1. Deparaffinize the paraffin sections to water:** Place the sections in sequence 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, wash with distilled water for several times, and wash clean.

**2. Staining with Glycine silver staining solution C:** Put the sections into the Glycine silver staining solution C for 5min, wash with distilled water for 3 times.

**3. Treatment with Glycine silver staining solution B:** Put the sections into Glycine silver

staining solution B for 3-5 minutes.

**4. Staining with Glycine silver staining solution A:** Take out the sections, quickly shake off the residual glycine silver staining solution B on the tissue, put it into glycine silver staining solution AI (preheated well at 45°C in advance), and observe the reduction effect at all times. Take out the sections after a few seconds and quickly put into the glycine silver staining solution A II (preheated at 45°C in advance). Quickly take out after a few seconds and wash with distilled water.

If the background of the sections is too dark, you can quickly treat it with glycine silver staining solution D for 1s, and wash with distilled water for 3 times.

**5. Dehydration and sealing:** 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.

### **III. Interpretation of results:**

Nerve axons, fibrils in nerve cells, neurofibrillary tangles, senile plaques and dendrites are black, background is golden yellow.

### **IV. Precautions:**

1. It must be cleaned with distilled water before and after treatment with glycine silver solution, otherwise impurities will easily appear.
2. Only one section can be stained at a time, thus it's easy to control the effect, and the reduction process must be rapid, the film is easy to be dirty if it is not controlled well.
3. All staining solutions need to be protected from light. The glycine silver solution can be used 3-5 times in the same day, the reducing solution should be renewed each time.