# 普鲁士蓝+DAB 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 |
| DAB | TCI | D0078 |
| 过氧化氢 | 国药集团化学试剂有限公司 | 10011208 |
| 中性树胶 | 国药集团化学试剂有限公司 | 10004160 |
| 亚铁氢化钾 | 阿拉丁 | P112418 |
| 盐酸 | 国药集团化学试剂有限公司 | 10011008 |
| 苏木素染液 | Wanwu | G1004 |
| 硫酸铝 | 国药集团化学试剂有限公司 | 1000118 |

## 二、试剂配制

DAB 染液 ： 0.1gDAB+100ml 的 0.01mol/L PBS

过氧化氢: 将 30%$H_2O_2$用 0.01mol/LPBS 稀释为 0.003%

DAB 显色液 : DAB 染液：0.003%的 $H_2O_2$=1:1

2%亚铁氢化钾: 亚铁氢化钾 2g，100ml 蒸馏水。

2%盐酸： 盐酸 2ml，蒸馏水 98ml。

**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 遍。

**2、普鲁士蓝染色：**将 2%亚铁氢化钾和 2%盐酸等比例混合，切片入混合液中染色 30min，蒸馏水洗 2 遍。

**3、DAB 染色：**DAB 显色液滴染约 5-10min，显微镜下控制显色程度，倾去染液用 0.01mol/L 的 PBS 溶液浸洗 1 次，蒸馏水洗 3 次

**4、染核：**苏木素染色 1min，自来水洗，盐酸水溶液分化，自来水洗，氨水水溶液返蓝，自来水洗。

**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。

**四、结果判读：**

组织中含有铁元素的部位呈棕褐色，细胞核呈浅蓝色，背景呈浅棕色或无色。

**五、注意事项：**

1、普鲁士蓝 DAB 染液要现配现用。

2、切片染色前用蒸馏水洗干净，防止自来水中的铁离子在染色中反应，造成假阳性。

3、在 DAB 的显色过程中，每隔约 5min 需要在显微镜下观察显色效果，由普鲁士蓝染出的弱阳性(即浅蓝色)被 DAB 替代形成棕色或者无蓝色的部位变成棕色即可。

# Perls staining + DAB 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

| Items | Manufacturer | Model |
|---|---|---|
| Absolute alcohol | Sinopharm Chemical Reagent Co., Ltd. | 100092683 |
| Xylene | Sinopharm Chemical Reagent Co., Ltd. | 10023418 |
| DAB | TCI | D0078 |
| Hydrogen peroxide | Sinopharm Chemical Reagent Co., Ltd. | 10011208 |
| Neutral balsam | Sinopharm Chemical Reagent Co., Ltd. | 10004160 |
| Potassium ferrocyanide | Aladdin | P112418 |
| Hydrochloric acid | Sinopharm Chemical Reagent Co., Ltd. | 10011008 |
| Hematoxylin dye | Wanwu | G1004 |
| Aluminum sulfate | Sinopharm Chemical Reagent Co., Ltd. | 1000118 |

2. Reagent preparation

DAB staining solution: 0.1g DAB + 100ml 0.01mol/L PBS

Hydrogen peroxide: Dilute 30% H2O2 with 0.01mol/LPBS to 0.003%

DAB color developing solution: DAB staining solution: 0.003% H2O2 = 1:1
2% Potassium ferrocyanide: 2g of potassium ferrocyanide, distilled water 100ml.
2% hydrochloric acid: hydrochloric acid 2ml, distilled water 98ml.

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, tap water washing and distilled water washing for 3 times.
(2) Prussian blue staining: mix 2% potassium ferrohydride and 2% hydrochloric acid in equal proportions, put slides into the mixed solution staining for 30 minutes, and wash twice with distilled water.
(3) DAB staining: staining with DAB color droplets for about 5-10 minutes, control the degree of color development under a microscope, after poured out the staining solution, rinse once with 0.01mol/L PBS solution an distilled water washing for three times .
(4) Nucleus staining: Hematoxylin dye staining for 1 min, tap water washing, differentiation with hydrochloric acid aqueous solution, tap water washing, immerse into aqueous ammonia solution and tap water washing.
(5) Dehydration and sealing: put the slides into absolute ethanol I 5min-absolute ethanol II 5min-absolute ethanol III 5min-xylene I 5min-xylene II 5min transparent, neutral balsam sealing.
(6) Microscope examination, image collection and analysis.

4. Results

The iron-containing parts of the tissue are brown, the cell nucleus is light blue, and the background is light brown or colorless.

5. Note

1. Prussian blue DAB staining solution should be ready for usage.
2. Washing the slides with distilled water before staining to prevent the ferri ion in the tap water from reacting during staining and causing false positive.
3. During the color development process of DAB, should observe color development effect under microscope every 5 minutes, until the weak positive stained by Prussian blue (ie light blue) is replaced by DAB to be brown or the part without blue turns brown.