髓鞘 LFB 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	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
髓鞘染液套装	Servicebio	G1030
伊红染液	Servicebio	G1002
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。

2、 髓鞘染色：髓鞘染液 A 置于 60℃ 烤箱预热 30min，切片入髓鞘染液 A 加膜加盖浸染 1h，取出切片快速自来水洗。

3、背景分化：切片浸入髓鞘染液 B 中稍分化 2s（趁热），直接浸入髓鞘染液 C 分化

15s，水洗终止分化，镜检，反复分化水洗和镜检，至髓鞘呈蓝色背景近无色即可;

4、复染伊红：切片入 65 度烤箱烤干（约 30min 至玻片干燥）拿出，冷却后进入 95%乙醇后后复染伊红。

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：

神经髓鞘呈蓝色，其他成分呈近乎无色。

四、注意事项：

1、将切片染完后，不能长时间放在水中清洗，颜色会褪掉。

2、注意交替分化时程度的把握，分过过度，髓鞘的颜色会褪掉，分化不足，则界限不清楚

3、若分化过度，可以将切片至于染液中重染。

Luxol Fast Blue (LFB) Staining Experimental Report

1. Lab equipment and reagents

1.1 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	Servicebio	
Upright optical microscope	Nikon Japan	Nikon Eclipse E100
Imaging system	Nikon Japan	NIKON DS-U3

1.2 Chemical Reagents

Items	Manufacturer	Model
Absolute alcohol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Luxol fast blue staining kit	Servicebio	G1030
Hematoxylin-Eosin solution	Servicebio	G1002
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

2. Experimental steps

2.1 Paraffin section deparaffinization and rehydration: put the slides into xylene I 20 minutes-xylene II 20 minutes-absolute ethanol I 5 min-absolute ethanol II 5 min-75% alcohol for 5 min, tap water washing.

2.2 Luxol fast bluestaining: placed luxol fast blue staining A is in a 60°C oven and preheated for 30 minutes. Put he slides intoluxol fast blue staining A and cover with membrane for 1 hour, then take out the slides quickly wash with tap water.

2.3 Background differentiation: put the slides into luxol fast blue staining B for 2s and directly immerse in luxol fast blue staining C for differentiation 15s, washing to terminate differentiation, microscope examination, repeated differentiation, washing and microscopic examination, until the myelin sheath is blue, the background is almost colorless;

2.4 Eosin counterstaining: put the slides in the oven at 65 degrees to dry it (about 30mins until the slides are dry) and take out and cool it, put into 95% ethanol and Eosin counterstaining.

2.5 Dehydration and sealing: put the slides into absolute ethanol I 5min-anhydrous ethanol II 5min-anhydrous ethanol III 5min-xylene I 5min-xylene II 5min -transparent, neutral balsam sealing.

2.6 Microscope examination, images collection and analysis.

3. Results

Nerve myelin sheath is blue, and other components are almost colorless.

4. Note

1. After stained, do not wash them in water for a long time due to the color will be fade.
2. Pay attention to the degree of alternating differentiation, if over-differentiation, the color of the myelin sheath will be fade, insufficient differentiation, the boundaries are unclear.
3. If over-differentiation, you can re-stain the slides in the staining solution.