神经锇酸染色实验报告

一、实验器材及试剂

1、实验器材

名称	厂家	型号
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
通用组织固定液	Wanwu	G1101
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
锇酸染液(原液)	Wanwu	
PB 缓冲液(原液)	Wanwu	
中性树胶	国药集团化学试剂有限公司	10004160

二、实验步骤

1、锇酸染色：将神经组织用固定液固定 24h 以上，切成一小块，流水冲洗 8h；将组织块用锇酸工作液(PB 缓冲液原液与锇酸原液 1:1 混合)室温避光浸染一夜。用巴氏吸管将锇酸染液吸出，倒入 PB 缓冲液反复吹打清洗 2 次后，加入新的 PB 缓冲液(PB 缓冲液原液与蒸馏水 1:1 混合)浸泡一夜。

2、脱水浸蜡：将组织块用蒸馏水洗 3 遍，50%、70%、80%、90%、100%I、100%II的乙醇依次浸泡，各 15min，二甲苯I15min，二甲苯II15min，浸蜡 3h 上，包埋，常规切片。

3、脱蜡封片：将烤好的切片放入二甲苯I20min，二甲苯II20min，二甲苯III透明 5min，中性树胶封片。

4、显微镜镜检，图像采集分析。

三、结果判读

1、神经髓鞘呈黑色圆环状，背景近无色。

四、注意事项

1、锇酸染色前后组织都必须流水冲洗干净，避免产生杂质；经锇酸染色的组织块尽量小，以便锇酸渗进组织。

2、锇酸工作液需要现配现用，染色过程中需要避光。

3、PB 缓冲液需要放置 4℃保存。

Osmium staining of nerve experimental report

1. Experimental equipment and reagents

1.1 Experimental equipment

Name	Manufactor	Model
Pathology slicer	Shanghai Leica Instrument Co., Ltd	RM2016
Frozen platform	Wuhan Junjie Electronics Co., Ltd	JB-L5
Tissue spreader	Zhejiang Kehua Instrument Co., Ltd	KD-P
Oven	Tianjin Laibo Rui Instrument Equipment Co., Ltd	GFL-230
Slides	Wanwu	
Orthostatic microscope	NIKON, JAPAN	NIKON ECLIPSE E100
Image system	NIKON, JAPAN	NIKON DS-U3

1.2 Main experimental reagents

Reagent name	Manufactor	Article number
Universal tissue fixative	Wanwu	G1101
Ethanol	Sinopharm Group Chemical Reagent Co. LtD	100092683
Xylene	Sinopharm Group Chemical Reagent Co. LtD	10023418
Osimic acid dye solution (stock solution)	Wanwu	
Buffer solution (PB)	Wanwu	
Neutral resin	Sinopharm Group Chemical Reagent Co. LtD	10004160

2. Experimental steps

2.1 Osmic acid staining: the nerve tissue was fixed with fixed solution for more than 24 h, cut into a small piece, washed with running water for 8 h; the tissue block was soaked in osmic acid working solution (Pb buffer solution and osmic acid solution 1:1 mixture) at room temperature and dark overnight. Osmic acid staining solution was sucked out by Babbitt pipette, and then it was blown into Pb buffer solution repeatedly for cleaning twice, and then soaked in new Pb buffer solution (Pb buffer stock solution mixed with distilled water 1:1) for one night.

2.2 Dehydration and wax dipping: the tissue blocks were washed with distilled water for three times, and then soaked in ethanol of 50%, 70%, 80%, 90%, 100% I and 100% II for 15 min, xylene I for 15 min, xylene II for 15 min, and wax immersion for 3 h, then embedded and conventional sectioning.

2.3 Three changes of pure ethanol for 5min，Two changes of pure xylene for 5 min transparent and then coverslip with neutral resin.

2.4 Observed under microscope and took images.

3. The results were as follows

3.1 The myelin sheath is black and circular, and the background is nearly colorless.

4. Precautions

4.1 The tissue before and after osmium acid staining must be washed clean with running water to avoid impurities; the tissue mass stained by osmium acid should be as small as possible so that osmium acid can infiltrate into the tissue.

4.2 Osmic acid working solution needs to be used now, and it needs to be protected from light in the dyeing process.

4.3 Pb buffer solution should be stored at 4 ℃.