未脱钙骨硬组织切片甲苯胺蓝染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
硬组织切片机	上海徕卡仪器有限公司	HistoCore AUTOCUT
组织摊片机	KEDEE	1 类
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
二甲苯	国药集团化学试剂有限公司	10023418
甲苯胺蓝染液	Wanwu	G1032
中性树胶	国药集团化学试剂有限公司	10004160
乙二醇乙醚乙酸酯	麦克林	E808814-2.5L

二、实验步骤

1、切片脱塑至水：依次将切片乙二醇乙醚乙酸酯I 6 h，37℃，乙二醇乙醚乙酸酯II过夜，37℃，乙二醇乙醚乙酸酯III室温 10-15 min，乙二醇乙醚乙酸酯IV室温 10-15 min，100%I乙醇 10 min，100%II乙醇 10 min，95%乙醇 10 min，90%乙醇 10 min，80%乙醇 10 min，自来水洗。

2、甲苯胺蓝染色：切片入染液 5 min，水洗，显微镜下控制染色程度，若染色较深用 95%乙醇分化，无水乙醇、二甲苯快速脱水、透明。

3、透明封片：切片入干净的二甲苯透明 5 min，中性树胶封片。

4、显微镜镜检，图像采集分析。

三、结果判读：

骨组织软骨呈紫蓝色，背景浅蓝色。

四、注意事项：

1、染完后的切片不能长时间放在水里，会褪色。

2、组织一定要完全烤干后再封片，避免组织残留小水珠。

Undecalcified bone Toluidine blue stain report

I. Experimental equipment and reagent

Equipment

Brand name	Manufactures	Model
Oven	Tianjin Leboree instrument equipment Co. Ltd	GFL-230
Microslide	Wanwu	
Optical Microscopy	NIKON	Nikon Eclipse E100
Imaging system	NIKON	Nikon DS-U3

Reagent

Reagent	Manufactures	Cat
Ethanol absolute	Sinopharm Chemical Reagent Co.,Ltd	100092183
Xylenes	Sinopharm Chemical Reagent Co.,Ltd	100023418
Toluidine blue staining solution	Wanwu	G1032
Neutral balsam	Sinopharm Chemical Reagent Co.,Ltd	10004160
Ethylene glycol ethyl ether acetate	Macklin	E808814-2.5L

II. Experiment procedure

1. Remove the embedding agent and rehydration

Put sections into ethylene glycol ethyl ether acetate I 6 h at 37 ℃ and ethylene ether acetate II overnight at 37 ℃. Then put them into ethylene glycol ethyl ether acetate III 10-15 minutes at room temperature and ethylene glycol ethyl ether acetateIV 10-15 minutes at room temperature. The sections were rehydrated in 100%I - 100%II - 95% - 90% - 80% alcohol. Each step takes anywhere for 10 minutes. Finally, rinse with running water.

2. Toluidine blue stain

The sections were put into the toluidine blue staining solution for 2 minutes. Then wash with water,

and the degree of staining was controlled under the microscope. 95% ethanol was used for differentiation if the staining was deeper. Anhydrous ethanol was rapidly dehydrated.

3. Transparent and mount

Put sections into three cylinders of anhydrous ethanol for 5 min and two cylinders of xylene for 5 min. Finally, mount the sections with neutral balsam.

4. Microscope observation and collection and analysis images.

III. Result determination

The cartilage is purplish blue with a light blue background.

IV. Matter need attention

1. After stain, the sections should not be kept in the water for a long time because the color will fade in the water.

2. Tissue must be completely dried and then mount the sections with neutral balsam.