未脱钙骨硬组织切片番红固绿染色实验报告

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
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
番红固绿(骨组织)染液套装	Wanwu	G1053
分化液	Wanwu	G1005-3
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、固绿染色：切片入固绿染液 3-4 min，水洗去多余染液，若颜色较浅复染一次，若颜色较深用分化液分化。

3、番红染色：切片入番红染液 15-30 s，三缸无水乙醇快速脱水。

4、透明封片：干净的二甲苯透明 5 min，中性树胶封片。

5、显微镜镜检，图像采集分析。

三、染色判读：

软骨呈红色或橙红色，背景绿色。

四、注意事项：

1、染色过程中，注意番红不能染过，易与绿色杂合呈紫蓝色。

2、若片子效果不佳，可用分化液分化水洗至颜色褪掉后重染。

Undecalcified bone Safranin O-Fast Green 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
Safranin O-Fast Green staining solution	Wanwu	G1064
Differentiation liquid	Wanwu	G1005-3
Blue returning liquid	Wanwu	G1005-4
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. Fast Green staining

Put sections into fast green staining solution for 3-4 minutes and washed with water. Stain again if the color is lighter. Otherwise, use the differentiation liquid to differentiate.

3. Safranin O staining

Put sections into safranin O staining solution for 15-30 s and three cylinders of anhydrous ethanol for rapid dehydration.

4. Transparent and mount

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

5. Microscope observation and collection and analysis images.

III. Result determination

The cartilage is red or salmon red with a green background.

IV. Matter need attention

1. During the dyeing process, note that it is easy to be mixed with green turn purple blue if safranin O stain excessive.

2. If effect of the result is less than satisfactory, the sections can be washed with differentiation liquid until the color fades and then stain again.