骨组织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
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**
环保型脱蜡透明液	Servicebio	**G1128**
番红固绿(骨组织)染液套装	Servicebio	**G1053**
分化液	servicebio	**G1040**
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：依次将切片放入环保型脱蜡透明液I20min-环保型脱蜡透明液II20min-无水乙醇I5min-无水乙醇II5min-75%酒精 5min，自来水洗。

2、固绿染色：切片入骨组织固绿染色液 1-5min，水洗去多余染液，至软骨呈无色，1%盐酸

酒精浸泡 10s，自来水稍洗。

3、番红染色：切片入骨组织番红染色液 1-5s，四缸无水乙醇快速脱水分别 5s、2s、10s、第四缸镜检。

4、透明封片：干净的二甲苯透明 5min，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，成骨呈绿色。有些结缔组织也呈红色。

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，可用盐酸酒精分化水洗至颜色褪掉后重染。

Safranin Fast Green Staining for Bone Experimental Report

1. Experimental Equipment and Reagents

1.1 Experimental equipment

Name	Manufactor	Model
Dehydrator	DIAPATH	Donatello
Embedding machine	Wuhan JunjieElectronics Co., Ltd	JB-P5
Pathological section machine	Shanghai Leica Instrument Co., Ltd	RM2016
Frozen platform	Wuhan Junjie Electronics Co., Ltd	JB-L5
KD-P Water Bath	Kedee	KD-P
Oven	Tianjin Lai Bo Rui Instrument Equipment Co., Ltd	GFL-230
Slides	Servicebio	
Orthostatic microscope	Nikon, JAPAN	Nikon Eclipse E100
Image system	NIKON, JAPAN	Nikon DS-U3

1.2 Main experimental reagents

Reagent name	Manufactor	Article number
Absolute ethanol	Sinopharm Group Chemical Reagent Co. LTD	100092683
Xylene	Sinopharm Group Chemical Reagent Co. LTD	10023418
BioDewax and Clear Solution	Servicebio	G1128
SaffronFastGreen dye solution kit(Bone Tissue)	Servicebio	G1053
Differentiation solution	Servicebio	G1040
Neutral resin	Sinopharm Group Chemical Reagent Co. LTD	10004160

2. Experimental Steps

2.1 Paraffin slides dewaxed as follow: Two changes of pure BioDewax and Clear Solution for 20 min. Two changes of pure ethanol for 5min. 75% ethanol for 5min. Keep slides in tap water.

2.2 Fast green staining: The slides stained in fast green dye solution for 1-5 minutes, washed away the excess dye solution until the cartilage was colorless, and soaked in 1% hydrochloric acid and alcohol for 10s. And then washing them with tap water.

2.3 Saffron staining: The slides were stained in saffron dye solution for 1-5s, and then put into four cylinders of absolute ethanol, where three for rapid dehydration for 5s, 2s, 10s respectively, and kept in the fourth cylinder.

2.4 The slides were immersed in xylene to transparent for 5min, sealing with neutral resin.

2.5 Observed under microscope, and took images for analysis.

3. **The Results Were As Follows**

Cartilage was red or orange-red, and bone formation was green. And some connective tissues were red.

4. **Precautions**

4.1 During the dyeing process, the saffron staining cannot be overtime, because it is easy to hybridize with fast green to become purple-blue.

4.2 If the result of the staining is not well, it can be washed with hydrochloric acid solution and alcohol until the color fades, and then re-stained.