



未脱钙骨硬组织切片天狼猩红染色实验报告

一、实验器材及试剂

1、实验器材

名称	厂家	型号
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
天狼猩红染液	Servicebio	G1018
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°C, 乙二醇乙醚乙酸酯II过夜, 37°C, 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、天狼猩红染色：切片入天狼猩红染液中染色 8-10 min, 两缸或三缸无水乙醇脱水, 各 3s、5s、30 s, 入正丁醇约 10 s。
- 3、脱水封片：切片再放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：

光学显微镜下胶原纤维红色, 背景黄色; 偏振光下, I型胶原呈橘黄色或亮红色的粗纤维, III型胶原呈绿色的细纤维。



武汉赛维尔生物科技有限公司

Wuhan Servicebio Technology Co., Ltd.

四、注意事项：

- 1、无水乙醇脱水时应控制程度，片子不能残留多余的染液，也不能影响黄色的背景。

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Undecalcified Bone Picrosirius Red Stain Report

1. Experimental equipment and reagent

1.1 Equipment

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

1.2 Reagent

Reagent	Manufactures	Cat
Ethanol absolute	Sinopharm Chemical Reagent Co.,Ltd	100092683
Xylenes	Sinopharm Chemical Reagent Co.,Ltd	100023418
Picrosirius Red staining solution	Servicebio	G1018
Neutral balsam	Sinopharm Chemical Reagent Co.,Ltd	10004160
Ethylene glycol ethyl ether acetate	Macklin	E808814-2.5L

2. Experiment procedure

2.1 Remove the embedding agent and rehydration

Put sections into ethylene glycol ethyl ether acetate I 6 h at 37 °C and ethylene ether acetate II overnight at 37 °C. Then put them into ethylene glycol ethyl ether acetate III 10-15 min at room temperature and ethylene glycol ethyl ether acetateIV 10-15 min 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.

2.2 Picrosirius Red staining

Put sections into picrosirius red staining solution for 8-10 min. Two or three cylinders of anhydrous ethanol were used for dehydration. Each step takes anywhere for 3, 5 and 30 s. Then about 10 s in n-butyl alcohol.



2.3 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 tissue section with neutral balsam.

2.4 Microscope observation and collection and analysis images.

2.5 Result determination

Collagen fibers appear red with a yellow background under optical microscope. Type I collagen is orange or bright red and type III collagen is green under polarized light.

3. Matter need attention

The degree of dehydration of anhydrous ethanol should be controlled. The sections should not retain excess stain solution or affect the color of background.