未脱钙骨硬组织切片 Von-kossa 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
组化笔	Gene tech	GT1001
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	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
Von-Kossa 染液套装	Servicebio	G1043
分化液	Servicebio	G1005-3
返蓝液	Servicebio	G1005-4
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，切片用超纯水浸洗 5 次；

2、用组化笔画圈圈住组织，切片放置于透明湿盒中，立即滴加 Von Kossa 染液于组织上，加盖，用紫外灯连续照射 5 min-20 min，蒸馏水洗多次。

3、苏木素染色：切片入苏木素染液染3-5 min，自来水洗，分化液分化，自来水洗，返蓝液返蓝，流水冲洗。

4、伊红染色：85%、95%的酒精梯度脱水各 5 min，入伊红染液 5 min；

5、脱水封片：切片依次放入无水乙醇 I 5 min，无水乙醇 II 5 min，无水乙醇III 5 min，二甲苯I 5 min，二甲苯II 5 min 透明，中性树胶封片。

6、显微镜镜检，图像采集分析。

三、染色判读：

钙盐沉积区域呈黑色或棕黑色，细胞核呈蓝色，背景呈红色。

四、注意事项：

1. Von Kossa染液滴染前，玻片必须用超纯水浸洗5次或以上；
2. 如果没有紫外灯，也可以用强太阳光照射；

Undecalcified Bone Von Kossa Stain Report

1. Experimental equipment and reagent

1.1 Equipment

Brand name	Manufactures	Model
Oven	Tianjin Leboree instrument equipment Co. Ltd	GFL-230
Microslide	Servicebio	
Tissue pencil	Gene tech	GT1001
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
Von kossa staining solution	Servicebio	G1043
Differentiation liquid	Servicebio	G1005-3
Blue returning liquid	Servicebio	G1005-4
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 ℃ 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.

2.2 Tissue was circled with organized strokes and sides in a transparent wet box.

Von kossa staining solution was immediately added to the tissue and ironed continuously with

ULTRAVIOLET lamp for 5-20 min. Finally, washed repeatedly with distilled water.

2.3 Nucleus staining

Put sections into the hematoxylin dyeing solution for 8-10 min, then washed with running water. Differentiated with differentiation liquid, then washed with running water. Use blue returning liquid to return blue.

2.4 Eosin staining

The sections were dehydrated with 85% and 95% ethanol absolute for 5 minutes respectively and dyed in eosin solution for 8-10 minutes.

2.5 Transparent and Mounting

Put sections into three cylinders of ethanol absolute for 5 minutes and two cylinders of xylene for 5 minutes. Finally, sealed the sections with neutral balsam.

2.6 Microscope observation and collection and analysis images.

3. Result determination

The calcium salt deposit area is black or brownish black, the nucleus is blue and the background is red.

4. Matter need attention

1. The sections must be dipped in ultra-pure water 5 or more times before Von Kossa drops are stain.

2. If you don't have UV lamp, you can exposure to strong sunlight.