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
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
Von-kossa 染色试剂盒	Servicebio	G1043
分化液	Servicebio	G1039
返蓝液	Servicebio	G1040
中性树脂	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：依次将切片放入二甲苯I20min-二甲苯II20min-无水乙醇I5min-无水乙醇II 5min-75%酒精 5min，自来水洗，蒸馏水洗三遍。

2、硝酸银反应：甩干切片上的水，用组画笔化圈后，切片滴加 Von Kossa 染液，用紫外灯连续照射 4 小时，蒸馏水洗多遍；

3、苏木素染色：切片入苏木素染液染3-5min，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 5min，入伊红染液 5min；

5、脱水封片：切片依次放入无水乙醇 I 5min -无水乙醇 II 5min-无水乙醇III5min -二甲苯I5min -二甲苯II5min 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、硝酸银溶液滴染前，切片必须用蒸馏水洗干净；

2、如果没有紫外灯，也可以用强太阳光照射。

Von-kossa Staining Experimental Report

1. Experimental Equipment and Reagents

1.1 Experimental equipment

Name	Manufactor	Model
Dehydrator	DIAPATH	Donatello
Embedding machine	Wuhan Junjie Electronics 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	Zhejiang Kehua Instrument Co., Ltd	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
Von-kossa dye solution kit	Servicebio	G1043
Differentiating solution	Servicebio	G1039
Ammonia	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 xylene for 20 min. Two changes of pure ethanol for 5min. 75% ethanol for 5min. And wash them by tap water for 3 times.

2.2 Silver nitrate reaction: After draining the slides, draw a circle around the tissues with PAP-Pen , and then drip Von Kossa dye solution on the slides. Continuously irradiated with UV lamp for 4 hours, and washed for several times with distilled water.

2.3 Hematoxylin staining: The slides were staining in hematoxylin dye solution for 3-5 minutes, rinse with tap water, and then differentiate with differentiating solution, rinse with tap water, and finally rinse with running water after returning to blue with ammonia .

2.4 Eosin staining: The slides were put into 85% and 95% alcohol gradient dehydration for 5min respectively, and stained in eosin dye solution for 5min.

2.5 Dehydration sealing: The slides are placed in three changes of absolute ethanol for 5min. Two changes of xylene for 5min. And sealed with neutral resin.

2.6 Observed under microscope and took images.

3. The Results Were As Follows

The calcium salt deposition area was black or brown-black, and the cell nucleus was blue, and the background was red.

4. Precautions

4.1 Before dripping the silver nitrate solution, the slides must be washed clearly with distilled water.

4.2 If there is no UV lamp available, it can also be irradiated with strong sunlight.