碱性磷酸酶（ALP）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	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
ALP 染液套装	Wanwu	G1033
苏木素染液	Wanwu	G1004
分化液	Wanwu	G1039
返蓝液	Wanwu	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：依次将切片放入二甲苯I20min，二甲苯II20min，无水乙醇I5min，无水乙醇II5min，75%酒精 5min，水洗，蒸馏水洗三遍。

2、工作液配制：碱性磷酸酶染色液 A 2.5ml，碱性磷酸酶染色液 B 2.5ml，碱性磷酸酶染色液 C 4.5ml，碱性磷酸酶染色液 D 0.2ml，蒸馏水 0.3ml 依次混合。

3、碱性磷酸酶染色液 F 使用方法：碱性磷酸酶染色液 F 液使用时需要 1：100 稀释（每 5ml 纯水中加入 1 滴浓的碱性磷酸酶染色液 F 即可，现配现用，未用完的弃掉）。

4、孵育染色：将工作液滴于组织切片上，切片放入湿盒中于 37℃孵育 4h，蒸馏水洗 2 遍，碱性磷酸酶染色液 E 处理 5min，蒸馏水洗 3-5 遍，碱性磷酸酶染色液 F 处理 30s-1min，流水冲洗 5min。

5、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 3s，自来水洗，返蓝液返蓝 3s，流水冲洗。

6、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。

7、显微镜镜检，图像采集分析。

三、结果判读

1、成骨细胞胞浆呈棕黑色，细胞核呈浅蓝色。

四、注意事项

1、工作液每配制一步都要混匀，现配现用。

2、染液应保存在 4℃，使用前先复常温。

Alkaline phosphatase (ALP) staining experiment 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
Pathology slicer	Shanghai Leica Instrument Co., Ltd	RM2016
Frozen platform	Wuhan Junjie Electronics Co., Ltd	JB-L5
Tissue spreader	Zhejiang Kehua Instrument Co., Ltd	KD-P
Oven	Tianjin Laibo Rui Instrument Equipment Co., Ltd	GFL-230
Slides	Wanwu	
Orthostatic microscope	NIKON, JAPAN	NIKON ECLIPSE E100
Image system	NIKON, JAPAN	NIKON DS-U3

1.2 Main experimental reagents

Reagent name	Manufactor	Article number
Ethanol	Sinopharm Group Chemical Reagent Co. LTD	100092683
Xylene	Sinopharm Group Chemical Reagent Co. LTD	10023418
Alkaline phosphatase dye	Wanwu	G1033
Hematoxylin dye	Wanwu	G1004
Differentiation liquid	Wanwu	G1039
Blue returning liquid	Wanwu	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 5 min, keep slides in tap water.

2.2 Working solution: 2.5 mL alkaline phosphatase dye solution A, 2.5 mL alkaline phosphatase dye solution B, 4.5 mL alkaline phosphatase dye solution C, 0.2 mL alkaline phosphatase dye solution D, mixing in 0.3 mL distilled water.

2.3 A drop of alkaline phosphatase dye solution F mixing in 5 mL pure water.

2.4 Incubation staining: the working solution was dropped on the slides, and the slides were incubated in a wet box at 37 ℃ for 4 hours,washed with distilled water twice , treated with alkaline phosphatase E solution for 5 min, washed with distilled water for 3~5 times, treated with alkaline phosphatase dye solution F for 30-60 seconds,and then washed in running water for 5 min.

2.5 The slides stained in hematoxylin dye solution for 3~5 min, and then washed in running water . Used 1% HCL solution to differentiate for 3 seconds, after rinsed in the tap water, rinsed in hydroxide for 5 seconds to turn blue. And then washed in running water.

2.6 Three changes of pure ethanol for 5min，Two changes of pure xylene for 5 min transparent and then coverslip with neutral resin.

2.7 Observed under microscope and took images.

3. The results were as follows

3.1 The cytoplasm of osteoblasts was brown black and the nucleus was light blue.

4. Precautions

4.1 Working solution should be used immediately after preparation.

4.2 The dye solution should be kept at 4 ℃ and then returned to normal temperature before use.