抗酒石酸酸性磷酸酶（Trap）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
TRAP 染液套装	Servicebio	G1050
苏木素染液	servicebio	G1004
分化液	servicebio	G1039
返蓝液	servicebio	G1040
中性树胶	国药集团化学试剂有限公司	10004160
ALP 染液套装	servicebio	G1033

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，蒸馏水洗三遍。

2、工作液配制：

Trap 孵育液配制：

注：将 TRAP 染液 B 与 TRAP 染液 C 等比例混合，现配现用。标记为 B 液备用。

将 TRAP 染液 D 20mg 用 TRAP 染液 E 1mL 溶解，标记为 C 液备用（C 液需提前至少 3 天配置）。

Trap 孵育液配制：TRAP 染液 A 18mL+B 液 1mL+C 液 1mL 混匀，再加 TRAP 染液 F 0.282g，充分溶解过滤后备用即为 TRAP 孵育液。

3、孵育染色：将切片用组画笔化圈后放在（加有一定量的防止切片蒸干的纯水）湿盒中，用蒸馏水 37℃孵育 2h，倾去蒸馏水，将切片重新放置在湿盒中，并滴加过滤好的现配的 trap 孵育液放于 37℃烤箱孵育 20min。

4、苏木素染色：倾去染液，水洗后，苏木素染液复染核 15s，分化液分化，返蓝液返蓝。

5、脱水封片：常规脱水，透明，中性树胶封片。

6、显微镜镜检，图像采集分析。

三、结果判读：

破骨细胞胞浆呈酒红色，核浅蓝色。

四、注意事项：

1、工作液配制过程中需充分溶解，现配现用。

2、染液应保存在 4℃，使用前先复常温。

Anti-Tartaric Acid Phosphatase (Trap) Staining 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
TRAP dye solution kit	Servicebio	G1050
Hematoxylin	Servicebio	G1004
Ammonia	Servicebio	G1040
Neutral resin	Sinopharm Group Chemical Reagent Co. LTD	10004160
ALP dye solution kit	Servicebio	G1033

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, and wash them by tap water for 3 times.

2.2 Reagent preparation:

Trap incubation solution preparation:

Note: Mix TRAP dye solution B and TRAP dye solution C in equal proportions. Mark it as B solution for future use.

Dissolve 20 mg TRAP dye solution D with 1 mL TRAP dye solution E, and mark it as C solution for spare (C solution needs to be prepared at least 3 days in advance)

Trap incubation solution preparation:

Mix 18mL TRAP staining A + 1mL B solution + 1mL C solution, and then add 0.282g TRAP dye solution F, fully dissolved and filtered for spare.

2.3 Incubation and staining: Put the slides in a circle with a PAP Pen in a wet box(add a certain amount of pure water to prevent the slides from drying), incubate with distilled water at 37°C for 2h, pour off the distilled water, and place the slides in the wet box again. Drip the filtered Trap incubation solution and put it in the oven at 37℃ for 20min.

2.4 Hematoxylin staining: After pouring the dye solution and washing it, use the hematoxylin solution to re-stain the nucleus for 15s. Use the differentiation fluid to differentiate, and ammonia to return blue.

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

2.6 Observed under microscope, and took images for analysis.

3. The results were as follows

The cytoplasm of osteoclasts showed wine red and the nucleus showed light blue.

4. Precautions

4.1 The working fluid needs to be fully dissolved in the process of preparation and ready for usage.

4.2 The dye solution should be stored at 4℃, and returned to normal temperature before usage.