β-半乳糖苷酶染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
冰冻切片机	Thermo	CRYOSTAR NX50
切片刀	上海徕卡仪器有限公司	LEICA 819
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
OCT 包埋剂	Sakura	4583
β-半乳糖苷酶染色液 A	Wanwu	G1073
β-半乳糖苷酶染色液 B	Wanwu	G1073
β-半乳糖苷酶染色液 C	Wanwu	G1073
β-半乳糖苷酶染色液 D	Wanwu	G1073
β-半乳糖苷酶染色液 E	Wanwu	G1073
β-半乳糖苷酶染色液 F	Wanwu	G1073
甘油明胶封片剂	Wanwu	G1402

二、染色步骤

1、工作液配制：染色液 A 940ul，染色液 B 10ul，β-半乳糖苷酶染色液 D50ul，依次加入，混匀即为工作液。

2、固定：取一张没经过固定的新鲜冰冻切片自然晾干，β-半乳糖苷酶染色液E固定15min，倾去固定液，蒸馏水洗3遍；

3、β-半乳糖苷酶染色：甩干片子上的水，将工作液滴于组织上，于37℃孵育16至18h。

4、 倾去工作液，蒸馏充分水洗3次。

5、β-半乳糖苷酶染色液F孵育3min后，水洗。

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。

三、结果判读：

衰老细胞呈蓝色。

四、注意事项：

1、标本需要是新鲜的冰冻切片或细胞，不能经过固定。

2、染液需要在-20℃的条件下储存，保质期一年。

3、工作液要现配现用，不可保存。

β-galactosidase Staining Experiment Report

I. Experimental equipment and reagents

1. Experimental equipment

Equipment name	Manufacturer	Model No.
Freezing microtome	Thermo	CRYOSTAR NX50
Slicing knife	Shanghai Leica Instrument Co., Ltd.	LEICA 819
Microscope slides	Wanwu	
Upright electron microscope	Japan NIKON	NIKON ECLIPSE E100
Imaging system	Japan NIKON	NIKON DS-U3

2. Main experimental reagents

Reagent name	Manufacturer	Item No.
OCT Embedding medium	Sakura	4583
β-galactosidase staining solution A	Wanwu	G1073
β-galactosidase staining solution B	Wanwu	G1073
β-galactosidase staining solution C	Wanwu	G1073
β-galactosidase staining solution D	Wanwu	G1073
β-galactosidase staining solution E	Wanwu	G1073
β-galactosidase staining solution F	Wanwu	G1073
Glycerin Jelly	Wanwu	G1402

II. Staining steps

1. Preparation of working solution: 940ul of staining solution A, 10ul of staining solution B, and 50ul of β-galactosidase staining solution D, add them in sequence and mix well, then the working

solution is made.

2. Fixation: Take a fresh frozen section that has not been fixed and let it dry naturally, fix it with β-galactosidase staining solution E for 15 min, pour out the fixation solution, and wash it three times with distilled water.

3. β-galactosidase staining: Spin off the water on the section, drop the working solution onto the tissue, and incubate at 37℃ for 16 to 18 hours.

4. Pour out the working solution and wash adequately with distilled water for 3 times.

5. After incubation with β-galactosidase stain F for 3 min, wash with water.

6. Dehydration and sealing: Put the sections in sequence into Anhydrous ethanol I for 5min - Anhydrous ethanol II for 5min - Anhydrous ethanol III for 5min - Xylene I for 5min - Xylene II for 5min for transparency, seal with neutral gum lastly.

III. Interpretation of results:

Senescent cells are blue.

IV. Precautions:

1. Specimens need to be fresh frozen sections or cells, and can't be fixed.

2. The staining solutions need to be stored at -20℃, and the shelf life is one year.

3. The working solution should be prepared freshly and use immediately, and cannot be stored.