石蜡切片组化 DABtunel 实验报告

一、实验原理

当细胞凋亡时，染色体 DNA 双链断裂而产生大量的粘性 3'-OH 末端，可在脱氧核糖核苷酸末端转移酶（TdT）的作用下，将脱氧核糖核苷酸和荧光素、过氧化物酶、碱性磷酸酶或生物素形成的衍生物标记到 DNA 的 3'-末端，从而可进行凋亡细胞的检测（TUNEL 法）。

二、实验器材及试剂

1、实验器材

名称	厂家	型号
脱水机	武汉俊杰电子有限公司	JT-12S
生物组织自动包埋机	武汉俊杰电子有限公司	JB-P5
石蜡包埋机（冷台）	武汉俊杰电子有限公司	JB-L5
转轮式切片机	徕卡显微系统上海有限公司	HistoCoreBIOCUT
组织摊片机	武汉俊杰电子有限公司	JK-5
烤箱	天津市莱玻特瑞仪器设备有限公司	GFL125
载玻片	海门市神鹰实验器材厂	188109
脱色摇床	武汉赛维尔生物科技有限公司	SYC-Z100
涡旋仪	武汉赛维尔生物科技有限公司	MX-F
掌上离心机	武汉赛维尔生物科技有限公司	DS-S 100
微波炉	美的	M1-L213B
组化笔	Gene tech	GT1001

移液枪	Dragon	KE003068
盖玻片	江苏汇达医疗器械有限公司	710510
显微镜	NIKON	ECLIPSE E100
江丰扫描仪	宁波江丰生物信息技术有限公司	KF-PRO-120

2、主要实验试剂

试剂	厂家	货号
无水乙醇	杭州宏达化工仪器有限公司	SJ003614
二甲苯	国药集团化学试剂有限公司	10023418
PBS 缓冲液	杭州浩克生物技术有限公司	HK0002
柠檬酸抗原修复液(9.0)	杭州浩克生物技术有限公司	HKI0004
DABtunel 试剂盒	杭州浩克生物技术有限公司	HKI0012
苏木素染液	杭州浩克生物技术有限公司	HK2053
苏木素分化液	杭州浩克生物技术有限公司	HK2054
苏木素返蓝液	杭州浩克生物技术有限公司	HK2055
中性树胶	国药集团化学剂有限公司	10004160

三、实验步骤

1. **石蜡切片脱蜡至水：**依次将切片放入二甲苯I 12min—二甲苯II 12min—无水乙醇I 6min—95%酒精 6 min—85%酒精 6 min，自来水洗 2 min。
2. **修复：**组织切片置于盛满柠檬酸抗原修复液（9.0）的修复盒中于微波炉内进行抗原修复，中火 8min，此过程中应防止缓冲液过度蒸发，切勿干片。自然冷却后将玻片置于双蒸水中在脱色摇床上晃动洗涤 3 次，每次 5 min。
3. **组织画圈：**切片稍干后沿着组织用组化笔沿着组织外围轮廓画一个与组织间隔 3-4 毫米的小圈，画完后用纯水洗涤 3 次，每次 5 min。

4. **加试剂：**按片子数量和组织大小取 tunel 试剂盒内适量试剂 TdT 酶 和白光反应液，按 1:100 混合，加到圈内覆盖组织，切片平放于湿盒内，37℃恒温孵育 1 小时，湿盒内加少量水保持湿度。
5. **HRP 二抗孵育：**切片用 PBS（PH7.4）洗涤 3 次，每次 5min。然后加 HRP 二抗工作液室温孵育 30 min，工作液浓度 HRP：PBST=1:500，洗涤后 DAB 显色。
6. **复染细胞核：**苏木素染色 2-3min 左右，自来水洗，分化液分化 2 秒，自来水冲洗，返蓝液返蓝 15-30s，流水冲洗。
7. **脱水封片：**将切片依次放入 75%酒精 4 min—85%酒精 4 min—无水乙醇I 4 min - 无水乙醇II 4 min—二甲苯I 4 min—二甲苯II 4 min 中脱水透明，将切片从二甲苯拿出来稍晾干，中性树胶封片。

四、结果判读

苏木素染出来的细胞核为蓝色，试剂盒标记棕色为阳性凋亡细胞核。

五、注意事项

1. 注意切片脱蜡是否彻底；
2. 实验过程中切片勿干片；
3. 添加 tunel 反应液之前需要换纯水洗涤。

DABTunel assay report (Paraffin section)

1. Experimental principle

When cells undergo apoptosis, the double strands of chromosomal DNA break, generating a large number of sticky 3'-OH termini. Under the action of terminal deoxynucleotidyl transferase (TdT), derivatives formed by deoxynucleotides and fluorescein, peroxidase, alkaline phosphatase, or biotin can be labeled to the 3'-terminus of DNA, thereby enabling the detection of apoptotic cells (TUNEL method).

2. Laboratory equipment and reagents

2.1 Laboratory equipment

Equipment	Manufacturers	Model
Dehydrator	Wuhan Junjie Electronics Co., Ltd	JT-12S
Paraffin embedding machine	Wuhan Junjie Electronics Co., Ltd	JB-P5
Frozen table	Wuhan Junjie Electronics Co., Ltd	JB-L5
Rotary microtome	Shanghai Leica Instrument Co., Ltd	HistoCoreBIOCUT
Tissue machine	Wuhan Junjie Electronics Co., Ltd	JK-5
Oven	Tianjin Leibo Terry Equipment Co., Ltd	GFL125
Glass microscope slides	Haimen Shenying Experimental Equipment Factory	188109
Rocker	Servicebio	SYC-Z100
Vortex	Servicebio	MX-F
Micro-centrifuge	Servicebio	DS-S 100

Microwave oven	Midea	M1-L213B
Liquid Blocker PAP Pen	Gene tech	GT1001
Pipettor	Dragon	KE003068
Coverslips	Jiangsu Huida Medical Instruments Co., Ltd	710510
Microscope	NIKON	ECLIPSE E100
Imaging System	Konfoong Bioinformation Tech Co.,Ltd	KF-PRO-120

2.2 Laboratory reagents

Reagents	**Manufacturers**	**Catlog**
Anhydrous ethanol	Hangzhou Hongda Chemical Instrument Co., LTD	SJ003614
Xylene	Sinopharm Chemical Reagent co., Ltd.	10023418
PBS solution	Hangzhou Haoke Biotechnology Co., Ltd	HK0002
Citric acid antigen repair solution (9.0)	Hangzhou Haoke Biotechnology Co., Ltd	HKI0004
DABtunel Assay Kit	Hangzhou Haoke Biotechnology Co., Ltd	HKI0012
Hematoxylin staining solution	Hangzhou Haoke Biotechnology Co., Ltd	HK2053
Hematoxylin differentiate solution	Hangzhou Haoke Biotechnology Co., Ltd	HK2054
Hematoxylin bluing solution	Hangzhou Haoke Biotechnology Co., Ltd	HK2055
Neutral gum	Sinopharm Chemical Reagent co., Ltd.	10004160

3. Experimental procedure

3.1 Dewaxing and hydration: Put the sections into xylene I 12 min - xylene II 12 min - anhydrous ethanol I 6 min - 95% Ethyl alcohol for 6 min - 85% Ethyl alcohol for 6 min, rinsing with tap water for 2 min.

3.2 Repair: The tissue sections were placed in a repair box filled with citric acid antigen repair solution (9.0), and the antigen repair was carried out in a microwave oven at medium heat for 8min. During this process, excessive evaporation of buffer solution should be prevented and the slices should not be dried. After natural cooling, the slide was placed in double steaming water and washed by shaking on the decolorizing table for 3 times, 5 min each time.

3.3 Draw a circle around the tissue: After the section is slightly dry, draw a small circle with a histochemical pen along the outer outline of the tissue with a distance of 3-4 mm from the tissue. After drawing, wash it with pure water for 3 times, 5 min each time.

3.4 Add reagents: Appropriate amount of reagent TdT enzyme and white light reaction solution were taken from tunel kit according to the number of slides and tissue size, mixed at 1:100, then added to the ring to cover the tissue, the slices were placed flat in a wet box, incubated at 37℃ for 1 hour, and a small amount of water was added to the wet box to maintain humidity.

3.5 HRP secondary antibody incubation: Sections were washed 3 times with PBS (PH7.4) for 5min each time. Then, HRP secondary antibody working liquid was added and incubated at room temperature for 30 min, the working liquid concentration HRP: PBST=1:500, and the color was developed by DAB after washing.

3.6 Restaining of nucleus: Hematoxylin restained the nuclei for 2-3min. Wash with tap water, hematoxylin differentiate solution differentiation for 2s, rinse with tap water, hematoxylin bluing solution back blue 15-30s, water rinse.

3.7 Dehydration and sealing: Put the sections into 75% Ethyl alcohol for 4 min - 85% Ethyl alcohol for 4 min - Anhydrous ethanol I for 4 min - Anhydrous ethanol II for 4 min-xylene I for 4 min - xylene II for 4 min, after slightly drying, , take the slices out of xylene for a little dry, and seal them with neutral gum.

4. Interpretation of results

Hematoxylin stained nuclei are blue, and DAB positive apoptotic nuclei are brownish yellow

5. Precautions

5.1 Pay attention to whether the slice dewaxing is thorough;

5.2 Don't let the slices dry out during the experiment;

5.3 Wash with pure water before adding tunel reaction solution.