石蜡切片荧光 tunel 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	海门市神鹰实验仪器厂	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
荧光显微镜	NIKON	ECLIPSE C1
3D 扫描仪	3D HISTECH	Pannoramic SCAN

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(6.0)	杭州浩克生物技术有限公司	HKI0001
AF488 Tunel 试剂盒	杭州浩克生物技术有限公司	HKI0010
DAPI	杭州浩克生物技术有限公司	HKI0005
抗荧光淬灭封片剂	杭州浩克生物技术有限公司	HKI0007-1

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. **微波修复:**切片放入装满柠檬酸抗原修复液(6.0) 的修复盒中，微波修复中火 8min 后置于室温自然冷切，双蒸水洗涤 3 次，每次 5min。
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 1:50 比例混合配制 tunel 工作液，TdT 酶 1 μl＋对应颜色反应液 50 μl 混合均匀；将 tunel 工作液加入到圈内覆盖组织，切片平放于湿盒内，37℃恒温

箱孵育 1.5 小时，湿盒内加少量水保持湿度。(浓度和孵育时间可以根据实验结果进行调节)。

5. **DAPI 染核**：切片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5 min。吸干圈内多余 PBS 再滴加 DAPI 染液，避光室温孵育 8min。
6. **封片**：切片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。将切片取出后用抗荧光淬灭封片剂封片。
7. **镜检拍照**：将切片置于荧光显微镜下观察并采集图像。（DAPI 紫外激发波长 361-389nm，发射波长 420nm，发蓝光；FITC 激发波长 465-495nm，发射波长 515-555 nm，发绿光；CY3 激发波长 540-580，发射波长 594nm，发红光）。

四、结果判读

DAPI 染出的细胞核在紫外的激发下为蓝色，tunel 试剂盒为绿色荧光素标记，阳性凋亡细胞核为绿光。

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。

FluorescenceTunel (Green) 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
3D Imaging System	3D HISTECH	Pannoramic SCAN

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 (6.0)	Hangzhou Haoke Biotechnology Co., Ltd	HKI0001
AF488 Tunel Assay Kit	Hangzhou Haoke Biotechnology Co., Ltd	HKI0010
DAPI	Hangzhou Haoke Biotechnology Co., Ltd	HKI0005
Anti-fluorescence quenching sealing tablets	Hangzhou Haoke Biotechnology Co., Ltd	HKI0007-1

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: Put the slices into a repair box filled with citric acid antigen repair solution (6.0), microwave repair at medium heat for 8min, then place them at room temperature for natural cold cutting, and wash them three times in double steaming water for 5min 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: Tunel working liquid was prepared by mixing at 1:50 ratio: 1 μl TdT enzyme + 50 μl corresponding color reaction liquid was mixed evenly; The tunel working liquid was added into the ring to cover the tissue, the slices were placed flat in a wet box, incubated at 37℃ for 1.5 hours, and a small amount of water was added to the wet box to maintain humidity. (The concentration and incubation time can be adjusted according to the experimental results).

3.5 DAPI restaining nuclei: The sections were placed in PBS (PH7.4) and washed by shaking on the decolorizing table for 3 times, 5 min each time. Blot the excess PBS in the ring and then add DAPI dye solution, and incubate at room temperature for 8min away from light.

3.6 Sealing: The sections were placed in PBS (PH7.4) and washed by shaking on the decolorizing table for 3 times, 5min each time. Remove the slices and seal them with anti-fluorescence quenching tablets.

3.7 Microscopy photography: Sections were observed under a fluorescence microscope and images were collected. (DAPI UV excitation wavelength 361-389nm, Emission wavelength 420nm, emitting blue light; The excitation wavelength of FITC is 465-495nm, and the emission wavelength is 515-555 nm, emitting green light; The excitation wavelength of CY3 is 540-580nm, and the emission wavelength is 594nm, emitting red light.

4. Interpretation of results

The nuclei stained by DAPI are blue under ultraviolet excitation, the kit is labeled with green fluorescein, and the positive apoptotic nuclei are green.

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.