冰冻切片荧光 tunel 实验报告

1.实验器材及试剂

1.1 实验器材

名称	厂家	型号
冰冻切片机	Thermo	Cryotome E
载玻片	wanwu	
盖玻片	江苏世泰实验器材有限公司	10212432C
脱色摇床	wanwu	TSY-B
涡旋混合器	wanwu	MX-F
掌上离心机	wanwu	D1008E
移液枪	Dragon	KE0003087/KA0056573
组化笔	wanwu	WG1066-1
冰箱	青岛海尔股份有限公司	BCD-192TGN
正置荧光显微镜	日本尼康	NIKON ECLIPSE C1
成像系统	日本尼康	NIKON DS-U3

1.2 主要实验试剂

试剂	厂家	货号
4%多聚甲醛	wanwu	G1101
PBS 缓冲液	wanwu	G0002
蛋白酶 K	wanwu	G1205
破膜液	wanwu	G1204
DAPI	wanwu	G1012
抗荧光淬灭封片剂	wanwu	G1401
Tunel 试剂盒	wanwu	G1502

合肥万物

2.冰冻切片荧光 tunel 实验步骤

2.1 冰冻切片固定：冰冻切片 37℃烘箱烘烤 10-20min，控干水分。置于 4%多聚甲醛固定 30min，于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。
2.2 蛋白酶 K 修复：切片稍甩干后用组化笔在组织周围画圈（防止液体流走），在圈内滴加蛋白酶 K 工作液覆盖组织，37 度温箱孵育 22min。将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。(蛋白酶 K 工作液配置方法，原液：PBS=1:9)
2.3 破膜：切片稍甩干后在圈内滴加破膜工作液覆盖组织，常温下孵育 20min，将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。(破膜液为 0.1%triton. 配置方法，triron 原液：PBS=1:1000)
2.4 室温平衡：切片稍甩干后在圈内滴加 buffer 覆盖组织，buffer 常温孵育 10min.
2.5 加反应液：按片子数量和组织大小取 tunel 试剂盒内适量 TDT 酶 ,dUTP，buffer 按 1:5:50 比例混合，加到圈内覆盖组织，切片平放于湿盒内，37℃恒温箱孵育 2 小时，湿盒内加少量水保持湿度。
2.6DAPI 复染细胞核：切片用 PBS（PH7.4）洗涤 3 次，每次 5min。去除 PBS 后在圈内滴加 DAPI 染液，避光室温孵育 10min。
2.7 封片：玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。切片稍甩干后用抗荧光淬灭封片剂封片。
2.8 镜检拍照：切片于荧光显微镜下观察并采集图像。（DAPI 紫外激发波长 330-380nm，发射波长 420nm，发蓝光；CY3 激发波长 510-561nm，发射波长 590 nm，发红光；

3.冰冻切片荧光 tunel 结果判读

DAPI 染出来的细胞核在紫外的激发下为蓝色，tunel 试剂盒为 CY3 荧光素标记，阳性凋亡细胞核为红色

（red）tunel assay protocol (frozen-slides)

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Freezing microtome	Thermo	Cryotome E
Glass microscope slides	wanwu	
Coverslips	CITOTEST	10212432C
Rocker	wanwu	TSY-B
Vortex	wanwu	MX-F
Micro-centrifuge	wanwu	D1008E
Pipettor	Dragon	KE0003087/KA0056573
Liquid blocker pen	wanwu	WG1066-1
Refrigerator	Haier	BCD-192TGN
Ortho-Fluorescent Microscopy	Nikon	NIKON ECLIPSE C1
Imaging system	Nikon	NIKON DS-U3

1.2 Major reagents

Name	Producer	Model
4% **paraformaldehyde**	wanwu	G1101
PBS solution	wanwu	G0002
Proteinase K	wanwu	G1205
Permeabilize solution	wanwu	G1204
DAPI	wanwu	G1012
anti-fade mounting medium	wanwu	G1401
Tunel assay kit	wanwu	G1502

2 Procedure

2.1 Fix frozen-slides: restore frozen-slides to room temperature and dry in air. Fix in cold acetone 10 min and then dry in air. Wash three times with PBS (pH 7.4) in a Rocker device, 5 min each
2.2 Antigen retrieval: eliminate obvious liquid, mark the objective tissue with liquid blocker pen. Add proteinase K working solution to cover objectives and incubate at 37℃ for 25 min. then wash three times with PBS (pH 7.4) in a Rocker device, 5 min each.（Method for configuring working solution of proteinase K, stock solution: PBS=1:9）
2.3 Permeabilization: eliminate excess liquid, add permeabilize working solution to cover objective tissue, then incubate at room temperature for 20 min. wash three times with PBS (pH 7.4) in a Rocker device, 5 min each.（The membrane breaking fluid is 0.1% triton. Configuration method, triron stock solution: PBS=1:1000）
2.4 Equilibrium at room temperature: After the slices are slightly dried, buffer is added to the tissues in the circle, and the buffer is incubated at room temperature for 10 minutes
2.5 Tunel reaction: Take appropriate amount of TDT enzyme, dUTP and buffer in the tunel kit according to the number of slices and tissue size and mix at 1:5:50 ratio。 Prepare this reaction solution according to demand before use. Add this mixture to objective tissue placed in a flat wet box, incubate at 37℃ for 2 h. be sure to keep the wet box moist by adding water
2.6 DAPI counterstain in nucleus: wash three times with PBS (pH 7.4) in a Rocker device, 5 min each. Then incubate with DAPI solution at room temperature for 10 min, kept in dark place.
2.7 Mount: wash three times with PBS (pH 7.4) in a Rocker device, 5 min each. Throw away liquid slightly, then coverslip with anti-fade mounting medium.
2.8 Microscopy detection and collect images by Fluorescent Microscopy. DAPI glows blue by UV excitation wavelength 330-380 nm and emission wavelength 420 nm;CY3 glows red by excitation wavelength 510-560 nm and emission wavelength 590 nmby excitation wavelength 465-495 nm and emission wavelength 515-555 nm;

3.Results

The nuclei stained by DAPI are blue under the excitation of ultraviolet light, the tune3 kit is labeled with CY3 fluorescein, and the positive apoptotic nuclei are red