石蜡切片荧光 tunel(绿光）实验报告

1.实验器材及试剂

1.1 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
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	上海慧泰仪器制造有限公司	DHG-9140A
载玻片	Servicebio	
盖玻片	江苏世泰实验器材有限公司	10212432C
脱色摇床	Servicebio	TSY-B
涡旋混合器	Servicebio	MX-F
掌上离心机	Servicebio	D1008E
移液枪	Dragon	KE0003087/KA0056573
组化笔	Servicebio	G6100
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
无水乙醇	国药集团化学试剂有限公司	100092183
环保型脱蜡液	武汉赛维尔生物	G1128
PBS 缓冲液	Servicebio	G0002
蛋白酶 K	Servicebio	G1205
破膜液	Servicebio	G1204
DAPI	Servicebio	G1012

抗荧光淬灭封片剂	Servicebio	G1401
Tunel 试剂盒	Servicebio	G1501

2.石蜡切片荧光 tunel 实验步骤

2.1 石蜡切片脱蜡至水：依次将切片放入二甲苯I10min-二甲苯II10min-二甲苯II10min-无水乙醇I5min-无水乙醇II5min-无水乙醇 III5min -蒸馏水洗。(冬天应该适当延长脱蜡时间）
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。
2.6 DAPI 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，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；FITC 激发波长 465-495nm，发射波长 515-555 nm，发绿光；

3.石蜡切片荧光 tunel 结果判读

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

(FITC) Tunel Assay Protocol (Paraffin-Slides)

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Dehydrator	DIAPATH	Donatello
Paraffin embedding machine	武汉俊杰电子有限公司	JB-P5
pathology slicer	Leica	RM2016
Frozen machine	武汉俊杰电子有限公司	JB-L5
Water bath-slide drier	浙江省金华市科迪仪器设备有限公司	KD-P
Oven	上海慧泰仪器制造有限公司	DHG-9140A
Glass microscope slides	Servicebio	
Coverslips	CITOTEST	10212432C
Microwave	Glanze	P70D20TL-P4
Rocker	Servicebio	TSY-B
Vortex	Servicebio	MX-F
Micro-centrifuge	Servicebio	D1008E
Pipettor	Dragon	KE0003087/KA0056573
Liquid blocker pen	Servicebio	G6100
Ortho-Fluorescent Microscopy	Nikon	NIKON ECLIPSE C1
Imaging system	Nikon	NIKON DS-U3

1.2 Major reagents

Name	Producer	Code
Ethanol	SCRC	100092183
Biodewax and Clear Solution	SERVICEBIO	G1128
PBS solution	Servicebio	G0002
Permeabilize solution	Servicebio	G1204

Proteinase K	Servicebio	G1205
DAPI	Servicebio	G1012
anti-fade mounting medium	Servicebio	G1401
Tunel assay kit	Servicebio	G1501

2. Procedure

2.1 Deparaffinize and rehydrate: incubate sections in 2 changes of xylene, 15-20 min each. Dehydrate in 2 changes of pure ethanol for 10 min each, followed by dehydrate in gradient ethanol of 95%, 90%, 80%, and 70% ethanol, respectively, 5 min each (extend deparaffinize time slightly in winter). Wash in distilled water.

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 Microscopic examination and collecting images through fluorescence microscope. DAPI emits blue light at an ultraviolet excitation wavelength of 330-380 nm and an emission wavelength of

420 nm; FITC has an excitation wavelength of 465-495 nm and an emission wavelength of 515-555 nm, and emits green.

3.Results

Nucleus is blue by labeling with DAPI. Tunel assay kit is labelled with FITC. Positive apoptosis cells are green.