冰冻切片 dab 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
显微镜	Nikon	E100
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
3%双氧水	wanwu	
苏木素染液	wanwu	G1004
苏木素分化液	wanwu	G1039
苏木素反蓝液	wanwu	G1040
中性树胶	国药集团化学试剂有限公司	**10004160**
Tunel 试剂盒	wanwu	G1507
DAB 显色剂	wanwu	G1212

2.冰冻切片 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 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 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。

2.3（可选步骤）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。

2.4 阻断内源性过氧化物酶：将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。切片放入 3%双氧水溶液，室温避光孵育 20 min，将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。

2.5 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.

2.6 加反应液： 去掉平衡液，Recombinant TdT enzyme：Biotin-dUTP Labeling Mix：Equilibration Buffer=1 μL：5 μL：50 μL 比例混合，加到圈内覆盖组织，切片平放于湿盒内，37℃温箱孵育 1 小时，湿盒内加少量水保持湿度。

2.7 加 Streptavidin-HRP 反应液：将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。 Streptavidin-HRP 和 TBST 按 1：200 比例混合，加到圈内覆盖组织，切片平放于湿盒内，37℃温箱孵育 30min。将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。

2.8 DAB 显色：切片稍甩干后在圈内滴加新鲜配制的 DAB 显色液，显微镜下控制显色时间，阳性为细胞核呈棕黄色，纯水冲洗切片终止显色。

2.9 复染细胞核：苏木素复染 1min 左右，纯水洗涤，分化液分化数秒，纯水洗涤，氨水返蓝，纯水冲洗。

2.10 脱水封片：将切片经 4 缸 100%酒精脱水，每缸 5min, 随后在正丁醇中浸泡 5min, 放入二甲苯中进行透明，时间 5min 以上，将切片从二甲苯拿出来稍晾干，中性树胶封片。

3.冰冻切片 tunel 结果判读

苏木素染细胞核为蓝色，DAB 显出来的阳性凋亡细胞核为棕黄色

DABTunel assay report (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
3% H2O2	wanwu	G0115
Hematoxylin staining solution	wanwu	G1004
Hematoxylin differentiate solution	wanwu	G1039
Hematoxylin bluing solution	wanwu	G1040
Resin mounting medium	SCRC	**10004160**

Tunel Assay Kit	wanwu	G1507
DAB reagent	wanwu	G1212

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 20 min. then wash three times with PBS (pH 7.4) in a Rocker device, 5 min each.

2.3 （optional step） 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.

2.4 Block endogenous peroxidase: wash three times with PBS (pH 7.4) in a Rocker device, 5 min each. Immerse in 3% H_2O_2 and incubate at room temperature for 20 min, kept in dark place. Then wash again three times with PBS (pH 7.4) in a Rocker device, 5 min each.

2.5 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

2.6 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 1 h. be sure to keep the wet box moist by adding water.

2.7 Reagent Streptavidin-HRP and TBST are mixed at a ratio of 1:200, added to the circle to cover the tissue, the slices are placed in a wet box, and incubated in a 37°C incubator for 30 minutes. The slides were placed in PBS (PH7.4) and washed with shaking on a decolorizing shaker 3 times, 5 min each time.

2.8 DAB developing: dry sections slightly, add fresh prepared DAB chromogenic reagent to marked tissue. Manage reaction time by observing in microscopy until nucleus shows brown-yellow. Then stop developing reaction by wash in pure water.

2.9 Counterstain in nucleus with Hematoxylin staining solution for 1 min and wash in pure water.

Treat with the differentiate solution for a few seconds, wash in pure water. Back to blue by bluing solution, wash in pure water.

2.10 Dehydrate the slices in 4 cylinders of 100% alcohol for 5 minutes each, then soak them in n-butanol for 5 minutes,Put it in xylene for transparency, more than 5min. Dry briefly and mount with resin mounting medium.

3. Results

Nucleus stained with hematoxylin are blue. The positive apoptosis cells developed by DAB reagent have brown-yellow nucleus.