细胞荧光 tunel 红光实验报告

1.实验器材及试剂

1.1 实验器材

名称	厂家	型号
载玻片	WANWU	
涡旋混合器	WANWU	MX-F
掌上离心机	WANWU	D1008E
脱色摇床	WANWU	TSY-B2.12.12.1
移液枪	Dragon	KE0003087/KA0056573
组化笔	WANWU	WG1066-1
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
PBS 缓冲液	WANWU	G0002
破膜工作液	WANWU	G1204
Tunel 试剂盒	WANWU	G1502
DAPI	WANWU	G1012
抗荧光淬灭封片剂	WANWU	G1401

2.细胞爬片荧光 tunel 实验步骤

2.1 细胞破膜：爬片稍甩干后用组化笔在盖玻片中间细胞分布均匀的位置画圈（防止抗体流走），加 50-100μl 破膜工作液，室温孵育 20min，PBS 洗 3 次，每次 5 min。

2.2 室温平衡：爬片稍甩干后在圈内滴加 buffer 覆盖组织，buffer 常温孵育 10min.

2.3 加反应液：按爬片数量大小取 tunel 试剂盒内适量 TDT 酶 ,dUTP，buffer 按 1:5:50 比例混合，加到圈内覆盖细胞，爬片置于 37℃恒温箱孵育 2 小时。

2.4 DAPI 复染细胞核：爬片用 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。

2.5 封片：爬片用 PBS（PH7.4）洗涤 3 次，每次 5min。爬片稍甩干后将有细胞的一面朝下

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用抗荧光淬灭封片剂将玻片封固在载玻片上封片。

2.6 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；CY3 激发波长 510-560，发射波长 590nm，发红光）。

3.细胞爬片荧光 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。

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(red) Tunel assay protocol (cell climbing slides)

1. Apparatus and reagents

1.1 Apparatus

Name	**Producer**	**Model**
Glass microscope slide	WANWU	
Vortex	WANWU	MX-F
Micro-centrifuge	WANWU	D1008E
Rocker	WA8NW	TSY-B
Pipettor	UDragon	KE0003087/KA0056573
Liquid blocker pen	WANWU	WG1066-1
Ortho-Fluorescent Microscopy	Nikon	NIKON ECLIPSE C1
Imaging system	Nikon	NIKON DS-U3

1.2 Major reagents

Name	**Producer**	**Model**
PBS solution	WANWU	G0002
Permeabilize solution	WANWU	G1204
DAPI	WANWU	G1012
anti-fade mounting medium	WANWU	G1401
Tunel assay kit	WANWU	G1502

2.Procedure

2.1 Permeabilization: dry the cell climbing slides slightly. Mark the objective area with liquid blocker pen, where add 50-100 μl of permeabilize working solution. Incubate for 20 min at room temperature. Wash three times with PBS solution, 5 min each.

2.2 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

2.3 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

2.4 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.

2.5 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.

2.6 Photomicrograph:

The sections were observed under a fluorescence microscope and images were collected. (DAPI ultraviolet excitation wavelength 330-380nm, emission wavelength 420nm, blue light emission; CY3 excitation wavelength 510-561nm, emission wavelength 590 nm, red light emission

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

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