细胞免疫荧光实验报告

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-B
移液枪	Dragon	KE0003087/KA0056573
组化笔	Wanwu 日本	WG1066-1
正置荧光显微镜	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	稀释比
PBS 缓冲液	Wanwu	G0002	
破膜工作液	Wanwu	G1204	
BSA	Wanwu	G5001	
一抗：			
二抗：			
DAPI	Wanwu	G1012	
抗荧光淬灭封片剂	Wanwu	G1401	

2. 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 血清封闭：在圈内滴加用 3%BSA 均匀覆盖组织，室温封闭 30min。（一抗是山羊来源用 10%驴血清封闭，一抗其它来源的用 3%BSA 封闭）

2.3 加一抗：轻轻甩掉封闭液，在细胞孔板里滴加 PBS 按一定比例配好的一抗，细胞培养板

平放于湿盒内 4° C 孵育过夜。

2.4 加二抗：细胞孔板置于脱色摇床上晃动洗涤 3 次，每次 5min。稍甩干后在圈内滴加与一抗相应种属的二抗覆盖组织，室温孵育 50min。

2.5 DAPI 复染细胞核：爬片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。切片稍甩干后在圈内滴加 DAPI 染液，避光室温孵育 10min。

2.6 封片：爬片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。玻片稍甩干后用抗荧光淬灭封片剂封片。

2.7 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. 免疫细胞化学结果判读

DAPI 染出来的细胞核在紫外的激发下为蓝色，阳性表达为相应荧光素标记的红光或者绿光

Immunofluorescence protocol (cell climbing slides)

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Glass microscope slides	Wanwu	
Vortex	Wanwu	MX-F
Micro-centrifuge	Wanwu	D1008E
Rocker	Wanwu	TSY-B
Pipettor	Dragon	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	Code	Dilution
PBS solution	Wanwu	G0002	
Permeabilize solution	Wanwu	G1204	
BSA	Wanwu	G5001	
Primary antibody			
Secondary antibody			
DAPI	Wanwu		
anti-fade mounting medium	Wanwu	G1401	

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 Block with serum: eliminate obvious liquid, mark the objective tissue with liquid blocker pen. Cover objective area with 10% donkey serum (for the case of primary antibody originated from goat) or 3% BSA (for the case of primary antibody originated from others) at room temperature

for 30 min.

2.3 Primary antibody: remove the blocking solution. Incubate cells with primary antibody (diluted with PBS appropriately) overnight at 4 ℃, placed in a wet box.

2.4 Secondary antibody: wash cell climbing slides three times with PBS (pH 7.4), placed in a Rocker device and 5 min each. Cover cell climbing slides with secondary antibody (appropriately respond to primary antibody in species) , incubate at room temperature for 50 min.

2.5 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.

2.6 Mount: wash three times with PBS (pH 7.4) in a Rocker device, 5 min each. Throw away liquid slightly. Put the slides on a glass microscope slide and then mount with anti-fade mounting medium.

2.7 Microscopy detection and collect images by Fluorescent Microscopy.：DAPI glows blue by UV excitation wavelength 330-380 nm and emission wavelength 420 nm; FITC glows green by excitation wavelength 465-495 nm and emission wavelength 515-555 nm; CY3 glows red by excitation wavelength 510-560 nm and emission wavelength 590 nm.

3 **Results**

Nucleus is blue by labeling with DAPI. Positive cells are green or red according to the fluorescent labels used.