细胞涂片免疫荧光实验报告

1. 实验器材及试剂

1.1 实验器材

名称	厂家	型号
盖玻片	江苏世泰实验器材有限公司	10212432C
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	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	稀释比
PBS 缓冲液	Wanwu	G0002	
破膜工作液	Wanwu	G1204	
BSA	Wanwu	G5001	
抗荧光淬灭封片剂	Wanwu	G1401	
一抗：			
二抗：			
DAPI	谷歌生物	G1012	
抗荧光淬灭封片剂	谷歌生物	G1401	

2. 免疫细胞化学实验步骤

2.1 细胞固定：细胞悬液，2800rpm 4℃离心 5min，弃上清液，根据底部沉淀的细胞量加入 2ml 4%多聚甲醛固定。若肉眼看不见细胞沉淀时，用 3000rpm/min，4℃离心 10min。

2.2 涂片制作：固定过的细胞悬液，2800rpm 25℃离心 5min，弃上清液，根据底部沉淀加

入 PBS：①若肉眼看不见细胞沉淀时，用 3000rpm/min，25℃离心 10min，依然无沉淀时，留取底部约 0.2ml 的液体，再加入 0.5ml 的 PBS 混匀后用移液枪吸取 200ul，滴于提前用组化笔画好的小圆圈中（3cmX2cm 的椭圆）；②若细胞沉淀量很少，绿豆大小时，弃上清，留取底部沉淀，加入 0.5mlPBS,用移液枪吹打混匀后，吸取 200ul，涂于提前用组化笔画好的小圆圈中（3cmX2cm 的椭圆)；③若细胞沉淀量很多，黄豆大小或更大时，弃上清，留取底部沉淀，加入 2mlPBS,用移液枪吹打混匀后，吸取 150ul，涂于提前用组化笔画好的大圆圈中（最大长边约 5cm，最大短边约 2cm 的椭圆），在显微镜下观察细胞量的多少，若还是过厚，再用 PBS 对倍稀释该细胞悬液，直至在显微镜下观察到细胞量涂布均匀。用枪将细胞悬液铺满整个圆圈，涂片放置自然晾干。

2.3 细胞固定和破膜：在圈内加 50-100μl 固定液。室温固定 20Min,后，洗掉固定液，加破膜工作液，室温孵育 20min，PBS 洗 3 次，每次 5 min。

2.4 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）

2.5 加一抗：轻轻甩掉封闭液，滴加 PBS 按一定比例配好的一抗，玻片平放于湿盒内 4° C 孵育过夜。

2.6 加二抗：玻片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组化试剂盒内与一抗相应种属的二抗覆盖细胞，室温孵育 50min。

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

2.8 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。玻片稍甩干后用抗荧光淬灭封片剂封片。

2.9 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. 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 (smear 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	
Resin mounting medium	Wanwu	G1403	
Primary antibody			
Secondary antibody		G1012	
DAPI	Wanwu		
anti-fade mounting medium	Wanwu	G1401	

2 Procedure

2.1 Cell fixation: The cell suspension was centrifuged at 2800rpm 4°C for 5min, discard the supernatant and add 2ml 4% paraformaldehyde to fix the cell suspension according to the amount of cells deposited at the bottom. If the cell can not be visible by naked eyes, centrifuge it with 3000 rpm/min at 4℃for 10 min.

2.2 Making smear slides: Centrifuged the fixed cell suspension with 2800 rpm/min at 25℃ for 5 min, discard the supernatant, add PBS according to the bottom sediment: and added PBS according to the precipitation at the bottom：① If the cell precipitation can not be seen by naked eyes, centrifuge it with 3000 rpm/min at 25℃ for 10 min, If there's still no precipitation, take 0.2 ml of liquid at the bottom, then add 0.5 ml of PBS and mix well, pipette 200ul, and drop it into a small circle (3cmX2cm oval) drawn with Liquid blocker pen in advance; ② If the amount of cell precipitation is small as a mung bean, discard the supernatant, take the precipitation at the bottom, add 0.5 ml of PBS. After mixing well with a pipette, take 200 ul to draw in the small circle (ellipse by 3 cm X 2 cm) drawn with Liquid blocker pen in advance. ③ If there's a lot of precipitation as the soybean or bigger, discard the supernatant, take the precipitation at the bottom, add 2 ml of PBS. After mixing well with a pipette, take 150 ul to draw in a large circle (ellipse by the maximum long edge about 5 cm and the maximum short edge about 5 cm) drawn with Liquid blocker pen in advance. Observe the amount of cells with the microscope. If it's still too much, dilute the cell suspension with PBS times until uniform cell volume coating can be observed under the microscope. Spread the cell suspension over the circle with a pipette placed to dry naturally.

2.3. Cell Fixation and Permeabilization: Add 50-100μl fixing solution inside the circle. When fixed at room temperature for 20 min, wash off the fixing solution, add permeabilize working solution, incubate at room temperature for 20 min, wash 3 times with PBS,for 5 min each time.

2.4 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.

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

2.6 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) labelled with HRP, incubate at room temperature for 50 min.

2.7 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.

2.8 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 resin mounting medium.

2.9 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.