冰冻切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
冰冻切片机	Thermo	Cryotome E
盖玻片	江苏世泰实验器材有限公司	10212432C
载玻片	Servicebio	
微波炉	格兰仕微波炉电器有限公司	P70D20TL-P4
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
冰箱	青岛海尔股份有限公司	BCD-192TGN
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
OCT 包埋剂	Servicebio	G6059-110ML	
EDTA（PH8.0)抗原修复液	Servicebio	G1206	
EDTA(PH9.0)抗原修复液	Servicebio	G1203	
柠檬酸（PH6.0）抗原修复液	Servicebio	G1202	
BSA	Servicebio	G5001	
4%多聚甲醛	Servicebio	G1101	
PBS 缓冲液	Servicebio	G0002	
自发荧光淬灭剂	Servicebio	G1221	
荧光一抗：			
荧光二抗：	Servicebio		
DAPI	Servicebio	G1012	
抗荧光淬灭封片剂	Servicebio	G1401	

2. 冰冻切片免疫荧光实验步骤

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 抗原修复：组织切片置于盛满 EDTA 抗原修复缓冲液（PH8.0）的修复盒中于微波炉内进行抗原修复。中火 8min，停火 8min，转为中低火 7min，此过程中应防止缓冲液过度蒸发，切勿干片。自然冷却后将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。（修复液和修复强度根据组织来确定）

2.3 画圈血清封闭：切片稍甩干后用组化笔在组织周围画圈（防止抗体流走），甩干 PBS，滴加 BSA，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）

2.4 加一抗：轻轻甩掉封闭液，在切片上滴加 PBS 按一定比例配好的一抗，切片平放于湿盒内 4° C 孵育过夜。（湿盒内加少量水防止抗体蒸发）

2.5 加二抗：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与一抗相应种属的荧光二抗覆盖组织，避光室温孵育 50min。

2.6 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。

2.7 淬灭组织自发荧光：玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。在圈内加入自发荧光淬灭剂 5min，流水冲洗 10min。

2.8 封片：切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. 冰冻切片免疫荧光实验结果判读

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 (Frozen-slides)

1. Apparatus and Reagents

1.1 Apparatus

Name	Producer	Model
Freezing microtome	Thermo	Cryotome E
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
Refrigerator	Haier	BCD-192TGN
Ortho-Fluorescent Microscopy	Nikon	NIKON ECLIPSE C1
Imaging system	Nikon	NIKON DS-U3

1.2 Major reagents

Name	Producer	Code	Dilution
OCT embedding medium	Servicebio	G6059-110ML	
EDTA antigen retrieval solution (pH 8.0)	Servicebio	G1206	
EDTA antigen retrieval solution (pH 9.0)	Servicebio	G1203	
Sodium citrate antigen retrieval solution (pH 6.0)	Servicebio	G1202	
BSA	Servicebio	G5001	
4% paraformaldehyde	Servicebio	G1101	
PBS solution	Servicebio	G0002	
Spontaneouss fluorescence quenching reagent	Servicebio	G1221	

Primary antibody		
Fluorescent-labelled secondary anyibody		
DAPI	Servicebio	G1012
anti-fade mounting medium	Servicebio	G1401

2. Procedure

2.1 Fix frozen-slides: Frozen slides are baked in a 37°C oven for 10-20 minutes to control the moisture. Fix in paraformaldehyde 30 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: Immerse the slides in EDTA antigen retrieval buffer (pH 8.0) and maintain at a sub-boiling temperature for 8 min, standing for 8 min and then followed by another sub-boiling temperature for 7 min. Be sure to prevent buffer solution evaporate. Let air cooling. Wash three times with PBS (pH 7.4) in a Rocker device, 5 min each. Use the right antigen retrieval buffer and heat extent according to tissue characteristics.

2.3 Circle and Serum blocking: Eliminate obvious liquid, mark the objective tissue with liquid blocker pen. Add 3% BSA to cover the marked tissue to block non-specific binding for 30 min .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)

2.4 Primary antibody: Throw away the blocking solution slightly. Incubate slides with primary antibody (diluted with PBS appropriately) overnight at 4 ℃, placed in a wet box containing a little water.

2.5 Secondary antibody: Wash slides three times with PBS (pH 7.4) in a Rocker device, 5 min each. Then throw away liquid slightly. Cover objective tissue with secondary antibody (appropriately respond to primary antibody in species), incubate at room temperature for 50 min in dark condition.

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 Spontaneous fluorescence quenching: Wash three times with PBS (pH 7.4) in a Rocker device, 5 min each . Add spontaneous fluorescence quenching reagent to incubate for 5 min. Wash in running tap water for 10 min.

2.8 Mount: Throw away liquid slightly, then coverslip with anti-fade 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.