冰冻切片 JC-1 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
微波炉	格兰仕微波炉电器有限公司	P70D20TL-P4
脱色摇床	北京市六一仪器厂	WD-9405A
涡旋混合器	天悦电子	TYXH-II
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
倒置荧光显微镜	日本尼康	NIKON ECLIPSE TI-SR
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	Wanwu	G6059-110ML	
PBS 缓冲液	Wanwu 碧云	G0002	
JC-1 检测试剂盒	天	C2006	1：500
DAPI	Wanwu	G1012	
抗荧光淬灭封片剂	Wanwu	G1401	

2. 冰冻切片免疫荧光实验步骤

2.1 画圈：冰冻切片在室温下复温，控干水分，组化笔画圈。

2.2 JC-1 孵育 ：在画好圈的组织上加入稀释好的 JC-1，37° 温箱孵育 20min。

2.3 染 DAPI:玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。滴加 DAPI

染液，室温避光孵育 10 分钟。

2.2 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。切片稍甩干后用抗荧光淬灭封片剂封片。

2.3 镜检拍照：切片于荧光显微镜下观察并采集图像。（紫外激发波长 330-380nm，发射波长 420nm；FITC 绿光激发波长 465-495nm，发射波长 515-555 nm；CY3 红光激发波长 510-560，发射波长 590nm）

3. 实验结果判读

红光和绿光为 JC-1 的阳性信号，蓝光为细胞核的着色。

Immunofluorescence staining report for jc-1 detection

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

1.2 Major reagents

Name	Producer	Code	Dilution
OCT embedding medium	Wanwu	G6059-110ML	
PBS solution	Wanwu	G0002	
Jc-1 staining kit	beyotime	C2005	1:500
DAPI	Wanwu	G1012	
anti-fade mounting medium	Wanwu	G1401	

2.Procedure

2.1 Circle : restore frozen slides to room temperature . eliminate obvious liquid, mark the objective tissue with liquid blocker pen.

2.2 JC-1 stain: Add JC-1 staining solution to the marked area, incubate at 37℃ for 20 min kept in dark place.

2.3 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.

2.4 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.

2.5 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. JC-1 positive cells labelled by fluorescein are red and green