冰冻切片 ROS 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
脱色摇床	Wanwu	TSY-B
涡旋混合器	Wanwu	MX-F
掌上离心机	Wanwu	D1008E
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
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	Wanwu	G6059-110ML	
PBS 缓冲液	Wanwu	G0002	
ROS 染液	SIGMA	D7008	1:500
DAPI	Wanwu	G1012	
自发荧光淬灭剂	Wanwu	G1221	
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，淬灭组织自发荧光：冰冻切片在室温下复温，控干水分。用组化笔在组织周围画圈（防止抗体流走），在圈内加入自发荧光淬灭剂 5min，流水冲洗 10min。

2.2 染色：在圈内滴加 ROS 染液， 避光恒温箱 37° 孵育 30min。

2.3 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。

2.4 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。

2.5 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，阳性表达为红光

Immunofluorescence staining report for ROS detection

(Frozen-slides)

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	
ROS staining solution	SIGMA	D7008	1:500
DAPI	Wanwu	G1012	
Spontaneouss fluorescence quenching reagent	Wanwu	G1221	
anti-fade mounting medium	Wanwu	G1401	

2. Procedure

2.1 Circle and Spontaneous fluorescence quenching: restore frozen slides to room temperature . eliminate obvious liquid, mark the objective tissue with liquid blocker pen. Add spontaneous fluorescence quenching reagent to incubate for 5 min. Wash in running tap water for 10 min.

2.2 Staining：add ROS staining solution to the marked area, incubate at 37℃ for 30 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. ROS positive cells labelled by fluorescein are red.