石蜡切片硫磺素染色实验报告

1. 实验器材及试剂

1.1 实验器材

名称	厂家	型号
脱水机	DIAPATH	Donatello
包埋机	武汉俊杰电子有限公司	JB-P5
病理切片机	上海徕卡仪器有限公司	RM2016
冻台	武汉俊杰电子有限公司	JB-L5
组织摊片机	浙江省金华市科迪仪器设备有限公司	KD-P
烤箱	上海慧泰仪器制造有限公司	DHG-9140A
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
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
无水乙醇	国药集团化学试剂有限公司	100092183	
二甲苯	上海凌峰化学试剂有限公司	1330-20-7	
PBS 缓冲液	Wanwu	G0002	
DAPI	Wanwu	G1012	
抗荧光淬灭封片剂	Wanwu	G1401	

2. 石蜡切片免疫荧光实验步骤

2.1 石蜡切片脱蜡至水：依次将切片放入二甲苯Ⅰ15min-二甲苯Ⅱ15min-无水乙醇Ⅰ5min-无水乙醇Ⅱ5min-85%酒精 5min-75%酒精 5min-蒸馏水洗。

2.2 硫磺素孵育：用 50%酒精配制，浓度为 0.3%，配好过滤，室温孵育 8min。

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。

3. 石蜡切片免疫荧光结果判读

DAPI 染出来的细胞核在紫外的激发下为蓝色，阳性表达为绿光

Paraffin Thioflavin staining protocol

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Dehydrator	DIAPATH	Donatello
Paraffin embedding machine	武汉俊杰电子有限公司	JB-P5
pathology slicer	Leica	RM2016
Frozen machine	武汉俊杰电子有限公司	JB-L5
Water bath-slide drier	浙江省金华市科迪仪器设备有限公司	KD-P
Oven	上海慧泰仪器制造有限公司	DHG-9140A
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
Ortho-Fluorescent Microscopy	Nikon	NIKON ECLIPSE C1
Imaging system	Nikon	NIKON DS-U3

1.2 Major reagents

Name	Producer	Code	Dilution
Ethanol	SCRC	100092183	
Xylene	上海凌峰化学试剂有限公司	1330-20-7	
PBS solution	Wanwu	G0002	
DAPI	Wanwu	G1012	
anti-fade mounting medium	Wanwu	G1401	

2 Procedure

2.1 Deparaffinize and rehydrate: incubate sections in 2 changes of xylene, 15 min each. Dehydrate in 2 changes of pure ethanol for 5 min, followed by dehydrate in gradient ethanol of 85% and 75% ethanol, respectively, 5 min each. Wash in distilled water

2.2 Thioflavin staining: Prepared with 50% alcohol at a concentration of 0.3%, filtered and incubated at room temperature for 8min.

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: 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. Thioflavin positive cells labelled by fluorescein are green.