石蜡切片 FJB 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
微波炉	格兰仕微波炉电器有限公司	P70D20TL-P4
脱色摇床	谷歌生物	TSY-B
涡旋混合器	谷歌生物	MX-F
掌上离心机	谷歌生物	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	

DAPI	Wanwu	G1012
中性树胶	国药集团化学试剂有限公司	10004160

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 滴加 FJB 工作液：用现配的 50%冰醋酸作溶剂，按 1:400 配置 FJB 工作液在画好圈的组织上加入稀释好的 FJB 绿色荧光探针，4° 过夜。

2.3 染核封片：DAPI 染核 8 分钟，纯水冲洗，用吹风机吹干，二甲苯透明 1min，中性树胶封片

2.4 镜检拍照：切片于尼康倒置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. 石蜡切片免疫荧光结果判读

蓝色标记的为细胞核，阳性表达为绿光 。

Paraffin FJB 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
Microwave	Glanze	P70D20TL-P4
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	
DAPI	Wanwu	G1012	
Resin mounting medium	Wanwu	G1403	

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 Dry sections slightly. Mark the objective tissue with fluid blocker pen in a circle. Use the currently prepared 50% glacial acetic acid as the solvent, and configure the FJB working solution according to 1:400. Cover the marked area with diluted fjb solution, incubate overnight at 4 ℃, placed in a wet box containing a little water.

2.3 DAPI counterstain in nucleus: wash three times with distilled water in a Rocker device, 5 min each. Then incubate with DAPI solution at room temperature for 10 min, kept in dark place. Rinse with distilled water , dry with a hair dryer, transparent xylene for 1min, and mount with resin mounting medium.

2.4 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. FJB positive cells labelled by fluorescein are green.