石蜡切片免疫荧光实验报告

一、实验器材及试剂

1. 实验器材

名称	厂家	型号
脱水机	俊杰电子有限公司	JJ-12J
包埋机	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	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	陕西依科生物技术服务有限公司	YK116
盖玻片	江苏世泰实验器材有限公司	10212432c
微波炉	美的微波电器制造有限公司	MM823LA6-NS
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	福州迈新	PEN-0002
正置显微镜	日本尼康	ci-s
扫描仪（白光、荧光）	3DHISTEC	PannoramicMIDI

2. 主要实验试剂

试剂	厂家
无水乙醇	国药集团化学试剂有限公司
二甲苯	国药集团化学试剂有限公司
柠檬酸（PH6.0)抗原修复液	陕西依科生物技术服务有限公司
PBS 缓冲液	陕西依科生物技术服务有限公司
BSA	BioFROXX 货号 EZ2811C238
一抗	客户提供
二抗	壮志生物
抗荧光淬灭剂	博士德
DAPI	MCE HY-D0814

二、免疫荧光染色实验原理及步骤

实验原理 免疫学的基本反应是抗原-抗体反应。由于抗原抗体反应具有高度的特异性，所以当抗原抗体发生反应时，只要知道其中的一个因素，就可以查出另一个因素。免疫荧光技术就是将不影响抗原抗体活性的荧光色素标记在抗体（或抗原）上，与其相应的抗原（或抗

体）结合后，在荧光显微镜下呈现一种特异性荧光反应。

免疫荧光染色实验步骤

1. **石蜡切片脱蜡至水：**依次将切片放入二甲苯Ⅰ20min-二甲苯Ⅱ20min-无水乙醇Ⅰ5min-无水乙醇Ⅱ5min-75%酒精 5min，自来水洗；
2. **抗原修复：**组织切片置于盛有柠檬酸（PH6.0)抗原修复液的高压锅内进行抗原修复，喷气计时 2.5min， 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；
3. **灭活：**0.3%甲醇过氧化氢灭活 15min，PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 6min；
4. **BSA 或者血清封闭:**切片稍甩干后用组化笔在组织周围画圈（防止抗体流走），在圈内滴加用5%BSA，室温封闭 1h；
5. **加一抗**轻轻甩掉封闭液，在切片上滴加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）；
6. **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8min。切片稍甩干后在圈内滴加与种属对应的二抗，覆盖组织，室温孵育 120min。之后玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 8min；
7. **DAPI 染核：**滴加DAPI，室温孵育 10min，之后玻片置于PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 6min；
8. **封片：**抗荧光淬灭剂封片；
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）。

三、染色结果判读：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 (paraffin-slides)

1 Apparatus and reagents

1. 1 Major apparatus

Name	Producer	Model
Dehydrator	Wuhan Junjie Electronics Co., Ltd	JJ-12J
Embedding machine	Wuhan Junjie Electronics Co., Ltd	JB-P5
Pathology slicer	Leica	RM2016
Frozen platform	Wuhan Junjie Electronics Co., Ltd	JB-L5
Organizer	KEDEE	KD-P
Oven	Shanghai Huitai Instrument Manufacturing Co., Ltd	DHG-9140A
Glass slide	Shaanxi Yike Biotechnology Co., Ltd	YK116
cover glass	Jiangsu Shitai Experimental Equipment Co., Ltd	10212432c
Microwave	Midea Microwave Electrical Appliance Manufacturing Co., Ltd	MM823LA6-NS
Rocker	Beijing Liuyi Instrument Factory	WD-9405A
Vortex	TianYue electronic	TYXH-II
Pipettor	Dragon	KE0003087/KA0056573
Liquid blocker pen	Fuzhou Maixin Biotechnology Co., LTD	PEN-0002
Upright optical microscope	Nikon	ci-s
Pathological panoramic digital scanner	3DHISTEC	PannoramicMIDI

1.2 Major reagents

Name	Producer	Code
Ethanol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Sodium citrate antigen retrieval solution (pH 6.0)	Shaanxi Yike Biotechnology Service Co., LTD	
PBS solution	Shaanxi Yike Biotechnology Service Co., LTD	
BSA	BioFROXX	
Primary antibody		

Fluorescent-labelled Secondary anyibody	Xi'an ambition Biotechnology Co., LTD
Spontaneouss fluorescence quenching reagent	BOSTER
DAPI	MCE

2 Procedure

2.1 Deparaffinize and rehydrate: incubate sections in 3 changes of Biodewax and Clear Solution,10 min each. Dehydrate in 3 changes of pure ethanol for 5 min. Wash in distilled water.

2.2 Antigen retrieval: The tissue sections were placed in a pressure cooker with citric acid (PH 6.0) antigen repair solution for antigen repair. Spray time for 2.5min. After natural cooling, the slides were placed in PBS (PH 7.4) and washed for 3 times for 5min each time.

2.3 Circle and Serum blocking: eliminate obvious liquid, mark the objective tissue with liquid blocker pen. Add 5% 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 5% 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,5min 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 cover slip with anti-fade mounting medium.

2.9 Microscopy detection and collect images by Fluorescent Microscopy. DAPI glows blue by UV excitation wavelength 330-380nm 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.