细胞 edu 染色荧光实验报告

1.实验器材及试剂

1.1 实验器材

名称	厂家	型号
载玻片	Wanwu	
涡旋混合器	Wanwu	MX-F
掌上离心机	Wanwu	D1008E
脱色摇床	Wanwu	TSY-B
移液枪	Dragon	KE0003087/KA0056573
组化笔	Wanwu 日本	WG1066-1
正置荧光显微镜	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
甘氨酸	Wanwu Wanwu	G5010-500G	
PBS 缓冲液	Wanwu	G0002	
破膜工作液	Wanwu Wanwu	G1204	
DAPI	锐博生物	G1012	
抗荧光淬灭封片剂		G1401	
EDU 染色试剂盒		C10310 、 C10338	

2.免疫细胞化学实验步骤

2.1 每孔加入 2 mg/mL 甘氨酸，脱色摇床孵育 5 分钟后，弃甘氨酸溶液；

注：作用是中和多聚甲醛，当采用其他方式进行细胞固定时可省略此步骤；

2.2 每孔加入 100 μL PBS，脱色摇床清洗 5 分钟，弃 PBS；

2.3 每孔加入 100μL 渗透剂(0.5% TritonX-100 的 PBS)脱色摇床孵育 10 分钟；PBS 清洗 1 次，5 分钟。

注：当实验需要进行其他抗体染色，或由于某些细胞类型对染料的吸附性较高，可能需要增强细胞膜通透性。

2.4 每孔加入 100 μL 的 1X Apollo®染色反应液（配置比例见表 3），避光、室温、脱色摇床孵育 30 分钟后，弃染色反应液；

2.5 加入 100 μL 渗透剂(0.5% TritonX-100 的 PBS) 脱色摇床清洗 2~3 次，每次 10 分钟，弃渗透剂；

2.6（加强）每孔每次加入 100 μL 甲醇清洗 1~2 次，每次 5 分钟；PBS 清洗 1 次，每次 5 分钟。

注：由于某些细胞对染料的吸附性较高，需采用加强方式洗脱以降低染料背景。

2.7 染核：爬片置于 PBS（PH7.4）中在脱色摇床上避光晃动洗涤 3 次，每次 5min。切片稍甩干后滴加 dapi 染液室温避光染核 10min。

2.8 封片：爬片用 PBS（PH7.4）洗涤 3 次，每次 5min。爬片稍甩干后将有细胞的一面朝下用抗荧光淬灭封片剂将玻片封固在载玻片上封片。

2.9 镜检拍照：切片于尼康正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.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

cell climbing slides edu staining protocol

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Glass microscope slides	Wanwu	
Vortex	Wanwu	MX-F
Micro-centrifuge	Wanwu	D1008E
Rocker	Wanwu	TSY-B
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
PBS solution	Wanwu	G0002	
Permeabilize solution	Wanwu	G1204	
Glycine	Wanwu	G5010-500G	
EDU staining kit	Wanwu	C10310	
DAPI	Wanwu	C10338	
anti-fade mounting medium	Wanwu	G1401	

2 Procedure

2.1 AAdd 2 mg/mL of glycine to each well, incubate in rocker device for 5 minutes, and discard the glycine solution.

Note: The action of this step is to neutralize paraformaldehyde, which can be omitted when Cell Fixation is performed in other ways.

2.2 Add 100μL of PBS to each well, wash in rocker device for 5 minutes, and discard PBS;

2.3 Add 100μL of penetrant to each well (0.5% TritonX-100 of PBS), incubate in rocker device for 10 minutes; wash with PBS once for 5 minutes.

Note: When the experiment requires other antibody staining, or because some cell types are highly adsorptive to dyes, it may be necessary to enhance cell membrane permeability.

2.4 Add 100 μL 1X Apollo® staining reaction solution to each well, and discard the staining reaction solution after incubating for 30 minutes at room temperature and protected from light;

2.5 Add 100 μL penetrant (0.5% TritonX-100 in PBS) and wash in a Rocker device 2 to 3 times, 10 minutes each time, discard the penetrant;

2.6 (Reinforced)add 100μL of methanol to each hole 1 or 2 times for 5 minutes each time; wash once with PBS for 5 min each time.

2.7 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.

2.8 Mount: Throw away liquid slightly, then coverslip with anti-fade mounting medium

2.9 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. Positive cells are green or red according to the fluorescent labels used.