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	Servicebio	
涡旋混合器	Servicebio	MX-F
掌上离心机	Servicebio	D1008E
脱色摇床	Servicebio	TSY-B
移液枪	Dragon	KE0003087/KA0056573
组化笔	Servicebio	G6100
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
PBS 缓冲液	Servicebio	G0002	
破膜工作液	Servicebio	G1204	
DAPI	Servicebio	G1012	
抗荧光淬灭封片剂	Servicebio	G1401	
EDU 染色试剂盒	武汉赛维尔生物	G1601	
		G1602	
		G1603	
		G1604	

2. 实验步骤

2.1 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。

2.2 每孔加入 100 μL 的 EDU 染色反应液（配置比例见下表），避光、室温、脱色摇床孵育 30 分钟后，弃染色反应液；

Component	Volume
反应缓冲液	935 μL
催化试剂（试剂A）	10 μL
荧光染料iF555（试剂B）	5 μL
催化添加剂（试剂C）	50 μL
总体积	**1000 μL**

2.3 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，或 hochest 染液室温避光染核 10min。

2.4 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。

2.5 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；555 红光激发波长 550nm，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	Servicebio	
Vortex	Servicebio	MX-F
Micro-centrifuge	Servicebio	D1008E
Rocker	Servicebio	TSY-B
Pipettor	Dragon	KE0003087/KA0056573
Liquid blocker pen	Servicebio	G6100
Ortho-Fluorescent Microscopy	Nikon	NIKON ECLIPSE C1
Imaging system	Nikon	NIKON DS-U3

1.2 Major reagents

Name	Producer	Code	Dilution
PBS solution	Servicebio	G0002	
Permeabilize solution	Servicebio	G1204	
Glycine	Servicebio	G5010-500G	
EDU staining kit	Servicebio	G1601 G1602 G1603 G1604	
DAPI	Servicebio		
Anti-fade mounting medium	Servicebio	G1401	

2. Procedure

2.1 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.

2.2 Add 100 μL EDU staining reaction solution to each well, and discard the staining reaction solution after incubating for 30 minutes at room temperature and protected from light;

Component	Volume
Reaction buffer	935ul
Catalytic reagent (A)	10ul
Fluorescent dye(B)	5ul
Catalytic additive(C)	50ul
Total volume	1000ul

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; 555 glows red by excitation wavelength 550 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.