



细胞鬼笔环肽（红光）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	
鬼笔环肽 (红光)	Servicebio	G1041	1: 200
DAPI	Servicebio	G1012	
抗荧光淬灭封片剂	Servicebio	G1401	

2. 实验步骤

2.1 细胞破膜：爬片稍甩干后用组化笔在盖玻片中间细胞分布均匀的位置画圈（防止抗体流失），加 50-100 μ l 破膜工作液，室温孵育 20min，PBS 洗 3 次，每次 5 min。

2.2 染色：在圈内滴加鬼笔环肽稀释液，避光常温孵育 2 小时。

2.3 DAPI 复染细胞核：爬片置于 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：爬片置于 PBS (PH7.4) 中在脱色摇床上晃动洗涤 3 次，每次 5min。玻片稍甩干后用抗荧光淬灭封片剂封片。

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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；CY3 激发波长 510-560，发射波长 590nm，发红光）。

3. 免疫细胞化学结果判读

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色。

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Phalloidine Staining Protocol for Cell Sample (Red)

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	
phalloidin (Cy3)	Servicebio	G1041	1:200
DAPI	Servicebio	G1012	
anti-fade mounting medium	Servicebio	G1401	

2. Procedure

2.1 Permeabilization: Dry the cell climbing slides slightly. Mark the objective area with liquid blocker pen, where add 50-100 μ l of permeabilize working solution. Incubate for 20 min at room temperature. Wash three times with PBS solution, 5 min each.

2.2 Phalloidine (Cy3) stain: Add phalloidine diluted solution to cover with the objective area, incubate at room temperature for 2 h in the dark.

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 in the dark.

2.3 Mount: Wash three times with PBS (pH 7.4) in a Rocker device, 5 min each. Throw away liquid slightly. Put the slides on a glass microscope slide and then mount with anti-fade mounting medium.



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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. Positive cells labelled by Phalloidine (Cy3) are red.