细胞爬片线粒体染色实验报告

1.实验器材及试剂

1.1 实验器材

名称	厂家	型号
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
PBS 缓冲液	Wanwu	G0002	
线粒体探针	Thermo Fisher Scientific	M7512	1:500
DAPI	Wanwu	G1012	
抗荧光淬灭封片剂	Wanwu	G1401	

2.细胞爬片线粒体染色实验步骤

2.1 细胞培养：于超净工作台内，打开六孔板，放置灭菌盖玻片。将细胞悬液滴加至盖玻片上，置于 CO_2浓度为 5%的培养箱中于 37℃培养至细胞固着（约 2h）。加入 2ml 细胞培养液继续培养约 6h。倒去培养基，用 PBS 洗 3 次，每次 5min。

2.2 探针孵育：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（防止抗体流走），加入用 PBS 稀释好的线粒体探针在避光条件下 37℃温箱孵育 30min。探针稀释比 1:500

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 hochest 染液室温避光染核 10min。（活细胞要用 hochest 染核，DAPI 染不上活细胞）

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；CY3 红光激发波长 510-560，发射波长 590nm）

3. 染色结果判读

细胞核为蓝色，阳性标记为红色荧光，根据细胞中的红色荧光来观察细胞线粒体含量和形态

Immunofluorescence staining report for mitochondria detection

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	
mitotracker	thermo	M7512	1:500
DAPI	Wanwu	G1012	
anti-fade mounting medium	Wanwu	G1401	

2 Procedure

2.1 Cell culture: In the ultra-clean workbench, open the six-well plate and place a sterilized cover slip. Drop the cell suspension onto the coverslip and place it in an incubator with a CO2 concentration of 5% at 37°C until the cells are fixed (approximately 2h). Add 2ml of cell culture medium to continue culturing for about 6h. Pour off the medium and wash 3 times with PBS for 5min each time.

2.2 Mitotracker stain : dry the cell climbing slides slightly. Mark the objective area with liquid

blocker pen, where add 50-100 μl of mitotracker working solution. incubate at 37℃ for 30 min kept in dark place.

2.3 Hoechst counterstain in nucleus: Wash three times with PBS (pH 7.4) in a Rocker device, 5 min each. Then incubate with hoechst solution at room temperature for 10 min in the dark.

2.4 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 resin 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; 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 mitotracker are red. Observe the content and morphology of l mitochondria according to the red fluorescence in the cell