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GF® 488 Phalloidin标记鬼笔环肽

Actin-Tracker Green-488 (微丝绿色荧光探针)

1. 产品简介:

鬼笔环肽（Phalloidin）是一种来源于毒蕈类鬼笔鹅膏（Amanita phalloides）的环状七肽毒素，以高亲和力（Kd= 20 nM）选择性结合于丝状肌动蛋白 F-actin，而不会与单体肌动蛋白 G-actin 结合，通常用来标记组织切片，细胞培养物或无细胞体系中的 F-actin，从而对 F-actin 进行定性和定量分析。另外，鬼笔环肽衍生物也以相近的亲和力结合于大小纤维，无论是动植物来源的肌肉细胞或非肌肉细胞，按照每一个肌动蛋白亚基约与一个鬼笔环肽分子的计量比结合。且非特异性结合几乎可忽略，染色区域和非染色区域辨识度非常明显。因此，鬼笔环肽衍生物特别适合替代肌动蛋白（Actin）抗体进行相关研究。另外鬼笔环肽衍生物很小，直径约 12-15Å，分子量＜2000 Daltons，未标记肌动蛋白（Actin）的许多生理特性都得以维持，比如，同肌动蛋白结合蛋白如肌球蛋白，原肌球蛋白，DNase I 等仍能发生反应；鬼笔环肽标记的纤维丝仍可穿透固相肌球蛋白基质；以及甘油抽提的肌纤维标记后仍可收缩等。

鬼笔环肽（Phalloidin）的结合阻止丝状肌动蛋白（微丝）的解离，稳定微丝结构，从而破坏微丝的聚合-去聚合的动态平衡。此特性使得肌动蛋白聚合发生的临界浓度（CC）降至＜1µg/mL，因此，可用作一种聚合促进剂。此外，鬼笔环肽还可抑制 F-actin 的 ATP 水解活性。

本品为 GF488 标记的鬼笔环肽，染色反应特异性强，对比性高，具有比 Actin 抗体更好的染色效果，适合用作 F-actin 的定性和定量检测。另外，经本品结合后的 F-actin 仍能维持 actin 自身具有的许多生物学特性。且本品的结合没有物种差异性，适用性广泛。本产品是冻干粉形式。

试剂盒组份:

Cat No	Product Name	Size	Price	Storage
JXF40152	GF 488 Phalloidin 标记鬼笔环肽 Actin-Tracker Green-488 (微丝绿色荧光探针)	300T	1200RMB	-20°C Protect from light 1 year
CAS#N/A		MW: ～1900		
最大激发/发射波长（Ex/Em）		Ex490nm; Em515nm		
外观（Appearance		黄色粉末（冻干粉）		
溶解性（Solubility）		溶于 DMSO、DMF、甲醇或者乙腈水溶液（20%）		
多肽序列（Sequence） GF488-bicyclic(Ala-DThr-Cys-cis-4-hydroxy-Pro-Ala-2-mercapto-Trp-4-hydroxy-5-amino-L eu)(S-3 to 6)				

操作步骤

1. 染色液的配置

1) 母液的配置：使用前将本品回温至室温并简短离心，加入 30uL DMSO 使其充分溶解，混匀即可获得 1000×GF488 标记鬼笔环肽母液。根据实验情况，对其分装并于-20°C避光干燥保存。

2) 工作液的配置：吸取 1 µL 以上 GF488 标记鬼笔环肽母液至 1 mL PBS（含 1%BSA）缓冲液中即可得到 1×工作液。

【注】：不同的细胞染色情况不同，相应 GF488 鬼笔环肽使用量也需根据不同情况而定。

2. 染色步骤

1）细胞爬片生长>24h，使其密度达到 50~60%汇合度。

2）吸掉培养液，37℃预热的 1×PBS（pH 7.4）清洗细胞 2 次。

3）使用溶于 PBS 的 4%甲醛溶液进行细胞固定，室温固定 10~30min。

注意：避免固定剂中含有甲醇成分，因为甲醇在固定过程中可能破坏肌动蛋白。

4）室温条件下，用 PBS 清洗细胞 2~3 次，每次 10min。

5）室温条件下，用丙酮（≤-20℃）脱水或者用 0.5% Triton X-100 溶液透化处理 5min。

6）室温条件下，用 PBS 清洗细胞 2~3 次，每次 10min。

7）取 100µL/孔（96 孔板）配制好的 GF488 标记鬼笔环肽工作液，覆盖住盖玻片上的细胞，室温避光孵育 30min（通常情况下，4℃~37℃孵育皆可）。

注意：为了降低背景，可于 GF488 标记的鬼笔环肽工作液内加入 1% BSA；另外，孵育过程中为了避免溶液挥发，可将盖玻片转移到一个密封的容器内。

8）用 PBS 清洗盖玻片 3 次，每次 5min。

9）使用 100 µL/孔（96 孔板）即用型 DAPI 溶液（浓度：100 nM）对细胞核进行复染，约 30s。

10）用 PBS 清洗盖玻片，然后倒置在已经滴有一滴 Fluoromount-GTM 水溶性封片剂的载玻片上。使用纸巾轻轻樑掉多余封片剂，然后用指甲油永久封片。

此法制备的标本玻片可置于 4℃避光保存，通常 6 个月内可继续做 F-actin 染色分析。

11）荧光显微镜或者共聚焦显微镜下进行荧光观察，选择 FITC 激发/发射滤片（Ex/Em=496/516nm）和 DAPI 激发/发射滤片（Ex/Em=364/454nm）。

需要自备材料

1） 甲醇
2） 1×PBS 缓冲液, pH 7.4, 细胞培养级别
3） 固定液 4%多聚甲醛（溶于 PBS 缓冲液）
4） 丙酮或透化液 0.5% Triton X-100（溶于 PBS 缓冲液）
5） Fluoromount-GTM 水溶性封片剂（不含 DAPI），DAPI
6） DAPI Fluoromount-GTM 水溶性封片剂（含 DAPI）
7） BSA，标准级别
8） 载玻片和盖玻片
9） 盖玻片周围密封液（如透明指甲油）
10）组装有 FITC 激发/发射滤片，以及 DAPI 激发/发射滤片的荧光显微镜或共聚焦显微镜。

注意事项：

1. 荧光标记鬼笔环肽的一个单位（T）的定义：按照推荐工作液浓度 200 nM，每次用量为 100 µL 染色工作液时，可以检测的次数 300 次；按照工作液浓度 100 nM，每次用量为 200 µL 染色工作液时，可以检测的次数也是 300 次。
2. 鬼笔环肽具有毒性，需小心操作。
3. 本产品为冻干粉形式，微量不易观察 使用前瞬时离心，加溶剂溶解后使用， 溶解后接近无色。
4. 产品仅限于专业人员用于生命科学研究，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅。
5. 产品必须由合格专业技术人员操作同时佩戴口罩/手套/实验服并遵守生物实验室安全操作规程！

GF® 488 Phalloidin标记鬼笔环肽

Actin-Tracker Green-488 (微丝绿色荧光探针)

1. Introduction

Phalloidin is a highly selective bicyclic peptide used for staining actin filaments (also known as F-actin). It binds to all variants of actin filaments in many different species of animals and plants. Typically, phalloidin is used conjugated to a fluorescent dye, such as FITC, Rhodamine, TRITC or similar dyes, such as GF® 488.

Phalloidin can be used with sample types such as formaldehyde-fixed and permeabilized tissue sections, cell cultures and cell-free experiments. It can also be used in paraffin-embedded samples that have been de-paraffinized. Importantly, phalloidin is also pH sensitive: at elevated pH, a key thioether bridge is cleaved, and the phalloidin loses its affinity for actin. Phalloidin staining can be combined with antibody-based staining by adding the phalloidin conjugate during the primary or secondary antibody incubation step.

phalloidin derivatives are convenient probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. Phalloidin binds to actin filaments much more tightly than to actin monomers, leading to a decrease in the rate constant for the dissociation of actin subunits from filament ends, essentially stabilizing actin filaments through the prevention of filament depolymerization. Moreover, phalloidin is found to inhibit the ATP hydrolysis activity of F-actin. Phalloidin functions differently at various concentrations in cells. When introduced into the cytoplasm at low concentrations, phalloidin recruits the less polymerized forms of cytoplasmic actin as well as filamin into stable "islands" of aggregated actin polymers, yet it does not interfere with stress fibers, i.e. thick bundles of microfilaments. The property of phalloidin is a useful tool for investigating the distribution of F-actin in cells by labeling phalloidin with fluorescent analogs and using them to stain actin filaments for light microscopy. Fluorescent derivatives of phalloidin have turned out to be enormously useful in localizing actin filaments in living or fixed cells as well as for visualizing individual actin filaments in vitro. Fluorescent phalloidin derivatives have been used as an important tool in the study of actin networks at high resolution. We offer a variety of fluorescent phalloidin derivatives with different colors for multicolor imaging applications.

Components of the kit:

Cat No	Product Name	Size	Price	Storage
JXF40152	GF 488 Phalloidin 标记鬼笔环肽 Actin-Tracker Green-488 (微丝绿色荧光探针)	300T	1200RMB	-20°C Protect from light 1 year
CAS#N/A		MW：～1900		
最大激发/发射波长（Ex/Em)		Ex490nm； Em515nm		
外观（Appearance		Amorphous solid		
溶解性（Solubility)		Soluble in DMSO, dimethyl formamide, methanol or water (warm).		
多肽序列（Sequence） GF488-bicyclic(Ala-DThr-Cys-cis-4-hydroxy-Pro-Ala-2-mercapto-Trp-4-hydroxy-5-amino-L eu)(S-3 to 6)				
Shipping: Ambient; Long Term Storage: -20°C Protect from light and moisture				

Protocol

1．PREPARATION OF WORKING SOLUTION

1) Stock preparation: Prepare 1000x stock solution by dissolving lyophilized phalloidin (300T) in 30uL DMSO. This stock solution can be sub-packaged then stored at -20°C. Solutions should be prepared **fresh** and **protected from light** when ever possible.

2）Working solution：Prepare 1x working solution by adding 1 µL of GF® 488-Phalloidin stock solution to 1 mL of PBS with 1% BSA.

Note: The stock solution of phalloidin conjugate should be aliquoted and stored at -20 °C. protected from light.

Note: Different cell types might be stained differently. The concentration of phalloidin conjugate working solution should be prepared accordingly.

2. SAMPLE EXPERIMENTAL PROTOCOL

Preparing culture of adherent cells

2.1 Grow cells in a 96 well black wall/clear bottom plate until they reach confluence (70–80%).

2.2 Cells can also be grown directly on coverslips inside a petri dish.

2.3 Aspirate cell culture medium (with care to avoid dislodging cells).

2.4 Wash once in PBS.

Tip: Avoid fixatives containing methanol or acetone: these disrupt the actin structure and prevent phalloidin staining.

Tip: Suspension cells can be attached to poly-D-lysine microplates or coverslips and then stained using the protocol for adherent cells.

Tip: Avoid fixatives containing methanol or acetone: these disrupt the actin structure and prevent phalloidin staining.

Alternative step: Preparing culture of cells in suspension

2.1 Grow cells until they reach desired confluence (70–80%).

2.2 Centrifuge cells at 1,000 rpm for 5 minutes and aspirate the supernatant, preserving the cell pellet.

2.3 Resuspend the cell pellets gently in pre-warmed (37°C) growth medium and transfer to microplate or coverslips.

2.4 Aspirate cell culture medium carefully to avoid dislodging cells. Wash once in PBS.

Tip: If you need to save time, suspension cells can be attached to poly-D-lysine microplates or coverslips and then stained using the protocol for adherent cells.

3. Stain cultured cells with phalloidin conjugates

Tip: Pre-incubating fixed cells with 1% BSA in PBS for 20–30 minutes may improve staining.

Tip: When staining coverslips, keep them in a covered container to minimize evaporation.

3.1 Fix cells in 3–4% formaldehyde in PBS at room temperature for 10–30 minutes.

3.2 Aspirate fixation solution and wash cells 2–3 times in PBS.

- **Tip:** Quench excess formaldehyde with 10 mM ethanolamine in PBS (or 0.1 M glycine in PBS) for 5 min.
- **Tip:** Add 0.1% Triton X-100 in PBS into the fixed cells for 3–5 minutes to increase permeability. Then wash cells 2–3 times in PBS.
- **Tip:** If cells do not appear healthy, add serum (2–10% range) to stain and wash solutions.

3.3 Add 100 µL/well (96-well plate) phalloidin-conjugate working solution. Incubate at room temperature for 20–90 minutes.

- **Tip:** add DNA staining dye at this point.
- **Tip:** for vertebrate cells, it may be possible to add phalloidin-conjugate to the final PBS wash and mount it in that medium.

3.4 To remove excess phalloidin conjugate，Rinse cells 2–3 times with PBS, 5 min per wash .

3.5 Add mounting media to preserve fluorescence (and seal to the slide if using coverslips).

3.6 Imaging under microscope with FITC filter set. Observe the cells at Ex/Em 490/515 nm.

Tip: A fast one-step approach to phalloidin staining is effective in some circumstances: a 20-minute incubation at 4ºC in 3.7% formaldehyde and 50–100 µg/mL lysopalmitoylphosphatidylcholine with phalloidin conjugate, followed by three washes and mounting.

NOTE: Always wear lab coats, gloves and goggles when working with our products although they are low-risk chemicals for R&D only.