# Fuorescent Phalloidin User Manual

| Catalog | Conjugate | Excitation | Emission |
|---|---|---|---|
| YS0016 | Phalloidin - AF488 (green) | 495 nm | 518 nm |
| YS0017 | Phalloidin - AF594 (red) | 593 nm | 614 nm |

## I. INTRODUCTION

Phalloidin is a bicyclic peptide that belongs to a family of toxins isolated from the deadly Amanita phalloides mushroom. Fluorescent phalloidins bind F-actin with nanomolar affinity and are water soluble, thus providing convenient probes for labeling, identifying, and quantifying F-actin in cryopreserved tissue sections, cell cultures, or cell-free experiments. Phalloidin contains an unusual thioether bridge between cysteine and tryptophan residues that forms an inner ring structure. At elevated pH, this thioether is cleaved and the toxin loses its affinity for actin.

Fluorescently labeled phalloidins stain F-actin at nanomolar concentrations. Labeled phalloidins have similar affinity for both large and small filaments, binding in a stoichiometric ratio of about one phalloidin molecule per actin subunit in muscle and non-muscle cells from various species of plants and animals. Different from antibodies, the binding affinity of phalloidin does not change significantly with actin among different species. Non-specific staining is negligible, and the contrast between stained and unstained areas is extremely large. Phalloidin shifts the monomer/polymer equilibrium toward the polymer, lowering the critical concentration for polymerization up to 30-fold. Phallotoxins also stabilize F-actin, inhibiting depolymerization by cytochalasin, potassium iodide and elevated temperatures. Because the phalloidin conjugates are small, with an approximate diameter of 12-15Å and molecular weight of <2000 Daltons, a variety of actin-binding proteins including myosin, tropomyosin and troponin can still bind to actin after treatment with phalloidin. Even more significantly, phalloidin-labeled actin filaments remain functional; labeled glycerinated muscle fibers still contract, and labeled actin filaments still move on solid-phase myosin substrates. Fluorescent phalloidin can also be used to quantify the amount of F-actin in cells.

## II. MATERIALS REQUIRED BUT NOT PROVIDED

1. Methanol
2. PBS (1X)
3. 4% Formaldehyde
4. 0.5% Triton X-100

## III. PROCEDURAL GUIDELINES

Handle fluorescent, biotinylated, and unlabeled phalloidins with care although the amount of toxin present in a vial could be lethal only to a mosquito (LD50 of phalloidin = 2 mg/kg).

## IV. WORKING SOLUTION PREPARATION

**Stock Solution**: Dissolve the lyophilized powder in 300 µl methanol for the 300 Assays size or 50 µl methanol for the 50 Assays size.

Dilute 1 µl fluorescent phalloidin stock solution in 200 µl PBS before use.
(For fluorescent phalloidins, the recommended dilution ratio is 1:40 - 1:200, one time experiment is equivalent to 1-5 µl stock solution in a total staining volume of 200 µl.)
*Note*: The dilution ratio can be adjusted appropriately according to the experimental effect.

## V. ASSAY PROCEDURE

**Staining fixed cells**

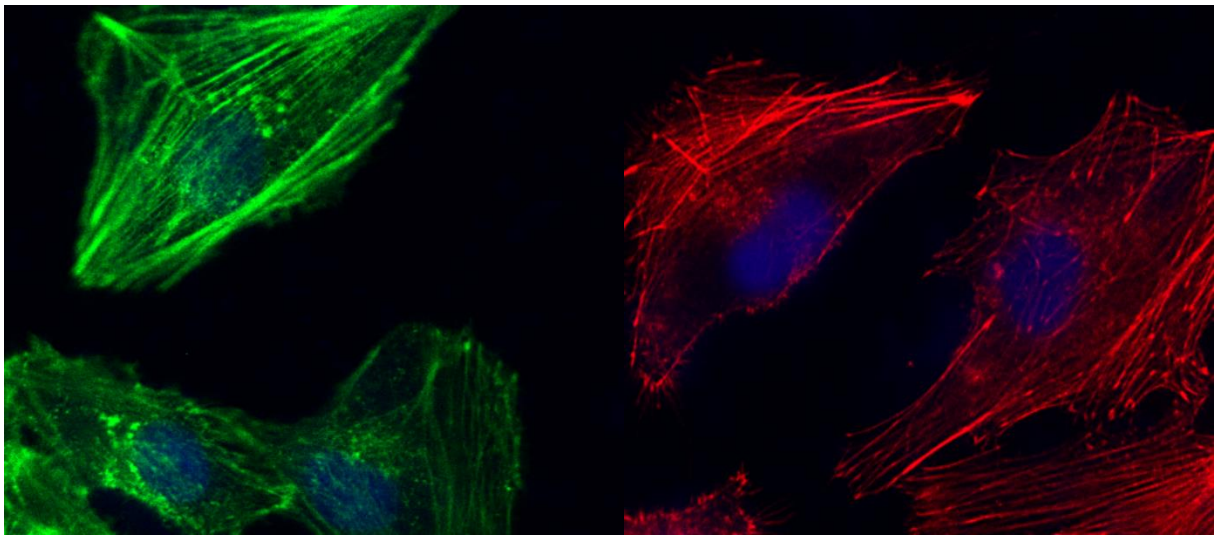
The following protocol describes the staining procedure for adherent cells grown on glass coverslips or 8-well chamber slides. Phalloidins also can be used to stain fixed frozen or paraffin tissue sections, as well as yeast and fungi.

1. Wash cells 3 times with PBS.
2. Fix cells on ice with 4% formaldehyde solution in PBS for 15 minutes.
Note: Methanol can disrupt actin during the fixation process. Therefore, it is best to avoid any methanol containing fixatives or other solvent-based fixatives. The preferred fixative is methanol-free formaldehyde.
3. Wash cells 3 times with PBS.
4. Permeabilize cells with 0.5% Triton X-100 in PBS at room temperature for 10 minutes.
5. Wash cells 3 times with PBS.
6. Dilute 1-5 µl fluorescent phalloidin stock solution in 200 µl PBS for each cover slip or chamber to be stained. Place the staining solution on the coverslip for 20 minutes at room temperature.
*Note*：Staining volume can be adjusted according to the sample. To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.
7. Wash 2-3 times with PBS.
8. Image using fluorescence microscopy. Fluorescent phalloidins are photostable enough to image in PBS, but for best results we recommend mounting with antifade mounting medium.

**Staining living cells**

Fluorescently-labeled phalloidin is not cell-permeant and has therefore has not been used extensively with living cells. However, living cells have been labeled by pinocytosis or

unknown mechanism. In general, a larger amount of stain will be needed for staining living cells. Alternatively, fluorescent phalloidins have also been injected into cells for monitoring actin distribution and cell motility.



Actin filamentswere stained with Phalloidin - 488(green) and 594 (red)

# 鬼笔环肽荧光系列说明书

| 货号 | 名称 | 激发光 | 发射光 |
|---|---|---|---|
| YS0016 | 鬼笔环肽- AF488 标记绿色 | 495 nm | 518 nm |
| YS0017 | 鬼笔环肽- AF594 标记红色 | 593 nm | 614 nm |

## 一、 引言

鬼笔环肽是从伞形毒蕈蘑菇中分离出来的一种毒素。它特异性结合于 F-肌动蛋白的双环肽。因此用荧光染料标记的鬼笔环肽可以非常方便的研究 F-肌动蛋白的分布。鬼笔环肽内部，在半胱氨酸和色氨酸之间含有不常见的硫醚桥形成内环结构。 在 pH 升高时，该硫醚被裂解，鬼笔环肽失去对肌动蛋白的亲和力。

荧光标记的鬼笔环肽可在纳摩尔水平染色 F-肌动蛋白。在各种植物细胞或动物细胞中，标记的鬼笔环肽对大、小细丝具有相似的亲和力，平均每个肌动蛋白亚基结合一个鬼笔环肽分子。不同于抗体，鬼笔环肽与肌动蛋白的结合亲和力在不同物种间没有显着变化。非特异性染色可以忽略不计，染色和未染色区域之间的对比度非常大。鬼笔环肽将单体/聚合物的平衡转向聚合状态，将聚合临界浓度降低至 30 倍。Phallotoxins 可通过抑制细胞松弛素的解聚，碘化钾和升高的温度，稳定 F-肌动蛋白。因为鬼笔环肽缀合物很小，大约直径 12-15 埃，分子量<2000 道尔顿，多种肌动蛋白结合蛋白，包括肌球蛋白，原肌球蛋白和后肌钙蛋白依然可以和鬼笔环肽标记的肌动蛋白结合。更重要的是，鬼笔环肽标记的肌动蛋白丝保持功能，标记甘油肌纤维仍然收缩，标记的肌动蛋白丝仍然可以继续移动。而且荧光标记的鬼笔环肽也可用于对细胞中 F-肌动蛋白进行定量研究。

## 二、需要但未提供的材料

1. 甲醇
2. PBS（1X）
3. 4%甲醛
4. 0.5%Triton X-100

## 三、 注意事项

本产品为冻干粉形式，使用前请瞬时离心，加适当溶剂溶解后使用。 -20℃干燥、避光保存，自收货之日起一年有效。若配制成水溶液，应小量分装保存。

## 四、 溶液制备

储备溶液：将冻干粉末溶解在 300μl 甲醇中，用于 300 次试验。
工作液：使用前，在 200μl PBS 中稀释 1μl 储备溶液成为工作液，建议现用现配
（对于荧光鬼笔素，建议的稀释比为 1:50-1:200，一次实验相当于总染色量为 200μl 的 1-5μl 储备溶液。）
注：稀释比可根据实验效果适当调整。

## 五、 使用步骤

**细胞染色**

以下方案是针对生长在玻璃盖玻片或 6 孔室玻片上的贴壁细胞的染色步骤。鬼笔环肽也可用于染色固定的冷冻或石蜡组织切片。

1. 用 PBS 清洗细胞 3 次。
2. 用含有 4％甲醛的 PBS 溶液固定细胞，冰上固定 15 分钟。

注意：甲醇可以在固定过程中破坏肌动蛋白。因此最好避免含有任何甲醇的固定剂。优选的固定剂是不含甲醇的甲醛。

3. 用 PBS 清洗细胞 3 次。
4. 用含 0.5％Triton X-100 的 PBS 溶液在室温下透化细胞 10 分钟。
5. 用 PBS 清洗细胞 3 次。
6. 用 200μL 工作液，加入一个盖玻片或孔中，室温孵育 20min，进行染色。

注：染色体积可根据样本情况进行调节。孵育过程中为避免染液挥发，可将盖玻片放于密封容器内。

7. 用 PBS 清洗细胞 2-3 次。
8. 荧光显微镜观察。此产品具有很好的光稳定，样品可以在 PBS 中成像，但为了效果最佳，也可以加入抗荧光淬灭剂观察。

**活细胞染色**

荧光标记的鬼笔环肽不具有细胞透性，因此没有被广范用于活细胞标记。然而，有报道称活细胞可能通过胞饮或未知机制进行标记。一般来说，染色活细胞时需要更多的染料。或者荧光标记的鬼笔环肽也可被注入到细胞中用于监测肌动蛋白分布和细胞运动。