Product Instruction Manual

Product Introduction

AF Phalloidin is an F-actin specific staining kit, with the core component being AF labeled phalloidin derivatives, designed specifically for cytoskeleton localization analysis experiments. This product binds to filamentous actin (F-actin) in eukaryotic cells with high affinity and specificity, without binding to monomeric G-actin, enabling precise labeling of cytoskeletal microfilaments. It binds to actin subunits at a 1:1 stoichiometric ratio and can be directly used for quantitative F-actin studies, while not affecting interactions between actin-binding proteins. It stabilizes the F-actin structure, prevents depolymerization, and fully preserves its biological function and physiological morphology. The accompanying next-generation AF fluorescent dyes offer comprehensive advantages over similar products in terms of brightness, photostability, and water solubility, and exhibit consistent affinity for microfilaments of different species and sizes. This product can be widely used for fluorescence microscopy (including laser confocal microscopy) observation and flow cytometry detection, making it suitable for applications such as cytoskeletal dynamics analysis, drug screening, and basic cell biology research. It provides researchers with a highly specific, highly stable, and efficient tool for cytoskeleton analysis.

Catalog Number

Cat	Name	Ex/Em
BGT-CHM-05132	AF488 Phalloidin	490/519 nm
BGT-CHM-05133	AF555 Phalloidin	555/565 nm

Storage and Shipping Conditions

Store at -20 °C protected from light. Expiration date is indicated on the outer package. Ship with ice packs.

Product Features

High Dye Specificity: AF Phalloidin binds specifically to F-actin with high affinity and does not recognize G-actin.

Compatible with Multiple Detection Platforms: Supports fluorescence microscopy observation and flow cytometry analysis.

Species-Independent: Unlike antibodies, the binding affinity of phalloidin to F-actin shows no significant variation across different species, and non-specific staining is essentially negligible.

Product Components

AF488 Phalloidin（BGT-CHM-05132）	
Components	Specifications
AF488 Phalloidin(1000×)	0.1 μL

AF555 Phalloidin（BGT-CHM-05133）	
Components	Specifications
AF555 Phalloidin(1000×)	0.1 μL

Precautions

1. Phalloidin has certain toxicity (LD50:2 mg/kg body weight). Please take appropriate protective measures when handling.
2. It is recommended to set up single-stained control groups in experiments for compensation adjustment during flow cytometry analysis.
3. The fluorescent dyes in the kit should be stored protected from light.
4. It is recommended to perform phalloidin staining after immunolabeling.
5. When staining yeast cultured in liquid medium, log-phase cells show significantly better staining results than stationary-phase cells.
6. This product is for research use only and must not be stored in ordinary residential areas.
7. For your safety and health, please follow the standard laboratory safety regulations of your institution.

Protocol

1. Pre-experiment Preparation

(1). Reagent Preparation:

a. Prepare sufficient PBS buffer, 4% paraformaldehyde fixative solution, and Triton X-100 (optional).

b. This product is a 1000× concentrate. The dilution ratio is 1:1000, add 0.1 μL of the stock solution to 1000 μL of PBS and mix well to obtain the working solution. Prepare immediately before use.

(2). Instrument Preparation:

Fluorescence microscope: Excitation/Emission wavelengths Ex/Em: 490/519 nm, 555/565 nm

(3). Cell Preparation：

Treat cells with corresponding drugs according to the experimental design.

2. Operating Procedures

Protocol 1: Fluorescence Microscopy (for Adherent Cells)

(1). Cell Treatment:

a.Seed cells in a multi-well plate one day in advance so that cell confluence reaches 70%–85%. After cells adhere, treat them according to the experimental design.

(2). Cell Washing:

a.Directly aspirate and discard the cell culture medium from the culture plate.

b.According to the plate specifications, add the corresponding volume of PBS to gently wash the cells 2~3 times (e.g., 100 μL/well for 96-well plates, 150 μL/well for 48-well plates, 250 μL/well for 24-well plates, 500 μL/well for 12-well plates, 1 mL/well for 6-well plates, 200 μL/well for 8-well chamber slides). After washing, aspirate the PBS completely.

(3). Cell Fixation:

a.Add an appropriate volume of 4% paraformaldehyde fixative solution and fix the cells at room temperature for 15 min. Wash with PBS 2–3 times.

Note: Methanol or other organic solvents can disrupt actin structures during fixation. It is recommended to use methanol-free formaldehyde as the fixative.

(4). Cell Permeabilization:

a.(Optional): At room temperature, permeabilize the cells with 0.4% Triton X-100 in PBS for 15 min to increase permeability.

b.Wash with PBS 2~3 times.

(5). Cell Staining:

a.Add the corresponding volume of staining working solution (AF488 Phalloidin) to each well (same volumes as in Step 2, e.g 100 µL/well for 96-well plates, 150 µL/well for 48-well plates, 250 µL/well for 24-well plates, 500 µL/well for 12-well plates, 1 mL/well for 6-well plates, 200 µL/well for 8-well chamber slides).

b.Incubate at room temperature in the dark for 15 min (incubation time can be optimized based on preliminary experiment results).

(6). Washing:

a.After incubation, aspirate and discard the staining working solution from each well.

b.Add PBS according to the volumes in Step 2 and wash the cells 2–3 times.

(7). Microscope Observation and Imaging:

a.Labeled phalloidin has good photostability. Samples can be imaged in PBS, but for best results, an anti-fading mounting medium can also be used.

Note: If the sample is not mounted, immediate imaging is strongly recommended. For optimal results, stained samples should be placed in an appropriate mounting medium and stored at 4°C protected from light.

Protocol 2: Live Cell Staining

Fluorescently labeled phalloidin is not cell-permeable and therefore has not been widely used for live-cell labeling. However, there are reports that live cells may be labeled through pinocytosis or unknown mechanisms. In general, more dye is required for staining live cells. Alternatively, fluorescently labeled phalloidin can be microinjected into cells to monitor actin distribution and cell motility.