Product Instruction Manual

Cat	Name	SIze	Ex/Em
BGT-CHM-05136	FITC Phalloidin Conjugate	1mg	495/519 nm

Storage and Shipping Conditions

Store at -20°C protected from light. Ship with ice packs.

Product Specifications

High dye specificity: Specifically binds to F-actin and does not recognize G-actin.

Compatibility with multiple detection platforms: Supports observation via fluorescence microscopy and analysis by flow cytometry.

No species limitation: Unlike antibodies, the binding affinity of phalloidin to F-actin shows no significant difference across species, and non-specific staining is almost negligible.

Product Introduction

FITC Phalloidin is a classic F-actin-specific staining reagent designed for cytoskeleton localization and morphological analysis. It specifically binds eukaryotic filamentous F-actin rather than monomeric G-actin, enabling precise microfilament labeling. This product binds actin subunits at a 1:1 stoichiometric ratio for quantitative F-actin detection without interfering with actin-binding protein interactions. It stabilizes F-actin structure, prevents depolymerization, and preserves native cytoskeleton morphology.Featuring high brightness, excellent water solubility and stable cross-species affinity, FITC dye adapts to various microfilament samples. Compatible with fluorescence microscopy, confocal microscopy and flow cytometry, this reagent is widely used for cytoskeletal dynamics analysis, drug screening and cell biology research, serving as a reliable, high-specificity tool for routine cytoskeleton staining.

Protocols

1.Pre-experiment Preparation

1.1 Reagent Preparation:

（1）Prepare sufficient PBS buffer, ddH_2O, methanol, methanol-free 4% paraformaldehyde fixative and Triton X-100 (optional).

（2）Stock Solution Preparation:

a. Centrifuge the tube briefly before dissolution. Dissolve the powder with either ddH_2O or methanol.

b. For 1mg package, add 90 mL solvent to prepare a stock solution at 200 T/mL.

Working Solution Preparation:

a. The recommended dilution ratio ranges from 1:40 to 1:200. Add 1–5 µL of 200 T/mL stock solution into 200 µL PBS and mix thoroughly.

1.2 Instrument Preparation

Fluorescence microscope excitation/emission wavelength: Ex/Em = 495/519 nm

1.3 Cell Preparation

Treat cells with corresponding drugs according to your experimental design.

2.Operating Procedures

Protocol 1: Fluorescence Microscopy Detection (For Adherent Cells)

2.1 Cell Treatment

（1）Seed cells into multi-well plates 24 hours in advance to reach 70%–85% confluence. After complete adherence, treat cells following the experimental plan.

2.2 Cell Washing

（1）Aspirate and discard cell culture medium directly from the culture plate.

（2）Add PBS of the corresponding volume to gently wash cells 2–3 times:96-well plate: 100 µL per well; 48-well plate: 150 µL per well; 24-well plate: 250 µL per well;12-well plate: 500 µL per well; 6-well plate: 1 mL per well; 8-well chamber slide: 200 µL per well.Completely aspirate PBS after washing.

2.3 Cell Fixation

（1）Add an appropriate volume of 4% paraformaldehyde fixative, fix cells at room temperature for 15 min, then wash 2–3 times with PBS.

Note: Methanol and other organic solvents will disrupt actin structure during fixation. Methanol-free paraformaldehyde is strongly recommended as the fixative.

2.4 Cell Permeabilization (Optional)

（1）Treat cells with 0.4% Triton X-100 (diluted in PBS) at room temperature for 15 min to increase membrane permeability.

（2）Wash cells 2–3 times with PBS.

2.5 Cell Staining

（1）Add staining working solution of the same volume used in the cell washing step:96-well plate: 100 µL per well; 48-well plate: 150 µL per well; 24-well plate: 250 µL per well;12-well plate: 500 µL per well; 6-well plate: 1 mL per well; 8-well chamber slide: 200 µL per well.

（2）Incubate at room temperature in the dark for 15 min; optimize incubation time based on preliminary experiments if needed.

2.6 Post-staining Washing

（1）Aspirate staining working solution after incubation.

（2）Wash cells 2–3 times with PBS using the same volume specified in the washing step.

2.7 Microscopy Imaging

（1）The labeled phalloidin possesses excellent photostability. Samples can be imaged directly in PBS; anti-fade mounting medium is recommended for optimal imaging effect.

Note: Image unfixed and unmounted samples immediately. For best preservation, mount stained samples with anti-fade mounting medium and store at 4 °C protected from light.

Protocol 2: Live Cell Staining

Fluorescently labeled phalloidin cannot penetrate intact cell membranes, so it is not commonly applied to live cell labeling. A small number of studies report that certain cell types may take up the dye via pinocytosis or other unknown pathways, which requires a higher dosage of staining reagent. Alternatively, fluorescent phalloidin can be microinjected into cells to monitor actin distribution and cell motility.

Precautions

1. Phalloidin is toxic (LD_{50} = 2 mg/kg body weight). Wear proper protective equipment during operation.
2. Prepare single-stained control groups for fluorescence compensation adjustment in flow cytometry assays.
3. The fluorescent dye in the kit must be stored away from light.
4. Perform phalloidin staining after immunolabeling for co-localization experiments.
5. For staining liquid-cultured yeast, log-phase cells yield far better results than stationary-phase cells.
6. This product is for research use only and must not be stored in residential premises.
7. Comply with standard laboratory safety regulations for your personal safety and health.