GeneCopoeia™
Expressway to Discovery

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Product Information

Rhodamine phalloidin

Catalog Number	Unit Size
C053	300 unit

Storage upon receipt:
- -20°C
- Protect from light

Product Description

Rhodamine phalloidin is a high-affinity F-actin probe conjugated to the red-orange fluorescent dye, tetramethylrhodamine (TRITC).

- **Selectively stains F-actin**
- **Excitation/Emission: 540/565 nm**
- **Superior to antibody staining**
- **Optimal for fixed and permeabilized samples**

Get Superior Results in Actin Staining Studies
Phalloidin is a bicyclic peptide belonging to a family of toxins isolated from the deadly *Amanita phalloides* "death cap" mushroom and is commonly used in imaging applications to selectively label F-actin. Fluorescently-labeled phalloidin has virtually identical binding properties with actin from different species including plants and animals. Phalloidin binds F-actin with high selectivity while fluorescein provides green fluorescence. Demonstrating very little nonspecific staining, fluorescein phalloidin allows high-contrast discrimination of actin staining.

Use in Multiple Applications

Fluorescein phalloidin can be used to visualize and quantitate F-actin in tissue sections, cell cultures, or cell-free preparations. Rhodamine phalloidin staining is fully compatible with other fluorescent stains used in cellular analyses.

Preparing the Stock Solution

The vial contents should be dissolved in 1.5 mL methanol to yield a final concentration of 200 units/mL, which is equivalent to approximately 6.6 µM.

One unit of **rhodamine phalloidin** is defined as the amount of material used to stain one microscope slide of fixed cells, according to the following protocol, and is equivalent to 5 µL of methanolic stock solution for the **rhodamine phalloidin**.

Stain Protocol

This procedure may not be optimum for a particular experimental system, but has yielded consistent results in most instances. The following protocol describes the staining procedure for adherent cells grown on glass coverslips.

Formaldehyde-Fixed Cells

1.1 Wash cells twice with prewarmed phosphate-buffered saline, pH 7.4 (PBS).

1.2 Fix the sample in 3.7% formaldehyde solution in PBS for 10 minutes at room temperature. **Note: Methanol can disrupt actin during the fixation process. Therefore, it is best to avoid any methanol containing fixatives. The preferred fixative is methanol-free formaldehyde.**

1.3 Wash two or more times with PBS.

1.4 Place each coverslip in a glass petri dish and extract it with a solution of acetone at ≦–20°C or 0.1% Triton X-100 in PBS for 3 to 5 minutes.

1.5 Wash two or more times with PBS.

1.6 Dilute 5 µL methanolic stock solution into 200 µL PBS for each coverslip to be stained. To reduce nonspecific background staining with these conjugates, add 1% bovine serum albumin (BSA) to the staining solution. It may also be useful to pre-incubate fixed cells with PBS containing 1% BSA for 20–30 minutes prior to adding the **rhodamine phalloidin** staining solution.

1.7 Place the staining solution on the coverslip for 20 minutes at room temperature. To avoid evaporation, keep the coverslips inside a covered container during the incubation.

1.8 Wash two or more times with PBS.

1.9 For long-term storage, the cells should be air dried and then mounted in a permanent mountant such as Cytoseal. Specimens prepared in this manner retain actin staining for at least six months when stored in the dark at 2–6°C.

Simultaneous Fixation, Permeabilization, and Rhodamine Phalloidin Staining

The **rhodamine phalloidin** appears to be stable for short periods in 4% formaldehyde fixation buffers. This permits a rapid one-step fixation, permeabilization, and labeling procedure as follows.
2.1 Prepare a 1 mL solution containing 50 to 100 µg/mL lysopalmitoylphosphatidylcholine and 3.7% formaldehyde and then add 5–10 units of **rhodamine phalloidin**.

2.2 Place this staining solution on cells and incubate for 20 minutes at 4°C.

2.3 Rapidly wash three times with buffer.

2.4 Mount coverslips and view.