

## Fluorescein 555-Phalloidin

Catalog No.: RA20011

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### Basic Information

Product name	Fluorescein 555-Phalloidin
Sizes	50T/300T
Storage	-20°C, keep away from light
Shipping	Shipped with ice pack
Validity	12 months

### Reagent Preparation

Fluorescein 555-Phalloidin: Dissolve the lyophilized powder in the tube with an appropriate amount of sterile water to prepare a 200T/mL stock solution (add 1.5mL of sterile water for 300T dye and 0.25 mL of sterile water for 50T dye).

One unit (T) of Fluorescein 555-Phalloidin is defined as the amount of dye needed to stain one slide loaded with cells. The recommended dilution ratio for Fluorescein 555-Phalloidin is 1:40-1:200, with one unit equal to 1-5  $\mu$ L of 200T/mL stock solution added to 200  $\mu$ L of total chromosome volume .

Note: The dilution ratio can be adjusted appropriately according to the actual staining effect.

### Operation Steps

#### Fixed cell staining

The following protocol is for staining adherent cells grown on glass coverslips or 8-well chamber slides . Phalloidin can also be used to stain fixed frozen tissue sections.

Staining paraffin tissue sections is not recommended.

(1) Wash cells three times with PBS.

(2) Fix the cells with PBS solution containing 4% formaldehyde at room temperature for 20 min.

NOTE: Methanol can damage actin during fixation. Therefore it is best to avoid fixatives that contain any methanol. The preferred fixative is formaldehyde, which does not contain methanol.

(3) Wash the cells three times with PBS.

(4) Permeabilize the cells with 0.4% Triton X-100 in PBS at room temperature for 10 min.

(5) Wash the cells three times with PBS.

(6) Dilute 1-5  $\mu$ L of fluorescently labeled phalloidin stock solution with 200  $\mu$ L PBS, add to a coverslip or well, and incubate at room temperature for 20 min for staining.

Note: The chromosome volume can be adjusted according to the sample conditions. To prevent the dye from evaporating during the incubation process, the cover glass can be placed in a sealed container.

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(7) Wash the cells 2-3 times with PBS.

(8) Fluorescence microscopy observation. The labeled phalloidin has good photostability, and the sample can be imaged in PBS, but for the best effect, an anti- fluorescence quencher can also be used for observation.

### **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 express phalloidin through endocytosis or unknown mechanisms.

In general, more dye is required to stain live cells. Alternatively, fluorescently labeled phalloidin can be injected into cells to monitor actin distribution and cell motility.

**Fluorescein 555 excitation/emission wavelength: 555/565nm**

### **Note**

1. All fluorescent dyes have quenching problems. Please try to avoid light to slow down fluorescence quenching.
2. To avoid repeated freezing and thawing, this product can be repacked into small quantities.
3. This product is For Research Use Only, Not for Diagnostic Use