



## Product Information

### ActinRed™ 555 Stain

Cat. No.	Product Name	Unit Size
C053	ActinRed™ 555 stain	300 unit

#### Spectral Properties:

Product Name	Ex (nm)	Em (nm)
ActinRed™ 555 stain	555	575

#### Storage upon receipt:

- -20°C
- Protect from light

#### Product Description

Our actin probes are prepared by conjugating phalloidin with our Andy Fluor dyes. These fluorescently-labeled phalloidins have virtually identical binding properties with actin from different species including plants and animals. These phalloidin conjugates maintain high binding affinity and selectivity with F-actin, providing useful probes for multicolor imaging applications.

#### Feature:

- High selectivity with F-actin
- Multicolor selection
- Good photostability
- Superior to antibody staining

#### Preparing the Stock Solution

Dissolve 300 unit of actin probe with 1.5 mL MeOH to make the stock solution of 200 units/mL.

One unit of actin probe 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  $\mu$ L of stock solution for the actin probe.

#### 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  $\leq -20^{\circ}\text{C}$  or 0.1% Triton X-100 in PBS for 3 to 5 minutes.
- 1.5 Wash two or more times with PBS.
- 1.6 Pre-incubate cells with PBS containing 1% BSA for 20–30 minutes.
- 1.7 Dilute 5  $\mu$ L stock solution into 200  $\mu$ L PBS with 1% BSA for each coverslip to be stained.
- 1.8 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.
- 1.9 Wash two or more times with PBS.
- 1.10 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 Actin Green Staining

The **actin probe** 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  $\mu$ g/mL lysopalmitoylphosphatidylcholine and 3.7% formaldehyde and then add 5–10 units of **actin probe**.
- 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.