GeneCopoeia™
Expressway to Discovery

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Product Information

Actin Stain

Cat. No.	Product Name	Unit Size
C051T	ActinBlue™ 350 stain	40 unit
C051	ActinBlue™ 350 stain	300 unit
C052T	ActinGreen™ 488 stain	40 unit
C052	ActinGreen™ 488 stain	300 unit
C053T	ActinOrange™ 555 stain	40 unit
C053	ActinOrange™ 555 stain	300 unit
C054T	ActinRed™ 594 stain	40 unit
C054	ActinRed™ 594 stain	300 unit
C055T	ActinFarRed™ 647 stain	40 unit
C055	ActinFarRed™ 647 stain	300 unit

Spectral Properties:

Product Name	Ex (nm)	Em (nm)
ActinBlue™ 350 stain	347	440
ActinGreen™ 488 stain	500	520
ActinOrange™ 555 stain	555	575
ActinRed™ 594 stain	590	614
ActinFarRed™ 647 stain	650	665

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 40 unit of actin probe with 200 µL MeOH or 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 µL 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 ≦–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 Pre-incubate cells with PBS containing 1% BSA for 20–30 minutes.

1.7 Dilute 5 µL stock solution into 200 µL 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 µg/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.