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Product Information

ActinGreen™ 488 Stain

Cat. No.	Product Name	Unit Size
C052	ActinGreen™ 488 stain	300 unit

Spectral Properties:

Product Name	Ex (nm)	Em (nm)
ActinGreen™ 488 stain	500	520

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 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.