IMMUNOLOGICAL SCIENCES Product Data Sheet

Alexa Fluor Dye Phalloidin Conjugates

Catalog #	Unit Size	Conjugate	Abs/Em (nm)
PP-10050	300 U	Phalloidin, Alexa Fluor 350	347/448
PP-10052	300 U	Phalloidin, Alexa Fluor 488	490/515
PP-10051	300 U	Phalloidin, Alexa Fluor 546	541/560
PP-10053	300 U	Phalloidin, Alexa Fluor 555	555/565
PP-10054	300 U	Phalloidin, Alexa Fluor 568	562/583
PP-10055	300 U	Phalloidin, Alexa Fluor 594	593/614
PP-10056	300 U	Phalloidin, Alexa Fluor 633	630/650
PP-10057	300 U	Phalloidin, Alexa Fluor 647	650/665
PP-10058	300 U	Phalloidin, Alexa Fluor 660	667/685
PP-10059	300 U	Phalloidin, Alexa Fluor 680	681/698

One unit of fluorescent phalloidin is defined as the amount of material used to stain one sample of fixed cells in a 200 uL volume (see protocols below).

Storage and Handling

Store at -20°C, desiccated, and protected from light. Lyophilized product is stable for at least one year from date of receipt when stored as recommended. After reconstitution in methanol or water, stock solutions are stable for at least one year when stored -20°C, protected from light. If using water as the solvent, freeze in aliquots. While the small amount of toxin in a vial is not likely to pose a heath hazard, it should be handled with care using universal laboratory safety precautions.

Important: See notes about the compatibility of specific Alexa Fluor dyes with fluorescence mounting media, and about the stability of phalloidin staining, after step 10 in the staining protocol (next page).

Product Description

Phalloidin is a toxin isolated from the deadly Amanita phalloides mushroom. It is a bicyclic peptide that binds specifically to F-actin (1). It is a very convenient tool to investigate the distribution of F-actin when labeled with fluorescent dyes.
Phalloidin contains an unusual thioether bridge between cysteine and tryptophan residues that forms an inner ring structure. At elevated pH, this thioether is cleaved and the toxin loses its affinity for actin.

Alexa Fluor dyes are a series of next-generation fluorescent dyes developed at Immunological Sciences to have combined advantages in brightness, photostability, and water solubility compared to other fluorescent dyes. Fluorescently labeled phalloidins stain F-actin at nanomolar concentrations (1-3). Labeled phalloidins have similar affinity for both large and small filaments, binding in a stoichiometric ratio of about one phalloidin molecule per actin subunit in muscle and non muscle cells from various species of plants and animals. Different from antibodies, the binding affinity of phalloidin does not change significantly with actin among different species. Non-specific staining is negligible, and the contrast between stained and unstained areas is extremely large. Phalloidin shifts the monomer/polymer equilibrium toward the polymer, lowering the critical concentration for polymerization up to 30-fold (3, 4). Phallotoxins also stabilize F-actin, inhibiting depolymerization by cytochalasins, potassium iodide and elevated temperatures. Because the phalloidin conjugates are small, with an approximate diameter of 12–15 Å and molecular weight of
<2000 Daltons, a variety of actin-binding proteins including myosin, tropomyosin and troponin can still bind to actin after treatment with phalloidin. Even more significantly, phalloidin-labeled actin filaments remain functional; labeled glycerinated muscle fibers still contract, and labeled actin filaments still move on solid-phase myosin substrates . Fluorescent phalloidin can also be used to quantify the amount of F-actin in cells.

Protocols

Preparation of Stock Solutions

Alexa Fluor dye phalloidin conjugates:

Dissolve the lyophilized solid in methanol or water (1.5 mL for the 300 U size or 0.25 mL for the 50 U size) to yield a stock solution of 200 U/mL.

One unit (U) of fluorescent phalloidin is defined as the amount of material used to stain one microscope slide of fixed cells. For fluorescent phalloidins one unit is equivalent to 5 uL of 200 U/mL stock solution in a total staining volume of 200 uL.

Staining Fixed Cells

The following protocol describes the staining procedure for adherent cells grown on glass coverslips or 8-well chamber slides. Phalloidins also can be used to stain fixed frozen or paraffin tissue sections, as well as yeast and fungi.

Note: When staining yeast in liquid culture, cells in log phase stain much bet- ter than cells in stationary phase.

1. Wash cells 3 times with PBS.
2. Fix cells on ice with 3.75% formaldehyde solution in PBS for 15 minutes.

   Note: Methanol can disrupt actin during the fixation process. Therefore, it is best to avoid any methanol containing fixatives or other solvent-based fixa- tives. The preferred fixative is methanol-free formaldehyde.
3. Wash 3 times with PBS.
4. Permeabilize cells with 0.5% Triton X-100 in PBS at room temperature for 10 minutes.
5. Wash cells 3 times with PBS.
6. Dilute 5 uL fluorescent phalloidin stock solution in 200 uL PBS for each cover slip or chamber to be stained. Volumes can be scaled as necessary depending on the size of the specimen or culture vessel.

   Note: For staining yeast or fungi, increasing the phalloidin concentration from 5 U/mL to 50 U/mL may improve penetration into the cells.
7. Place the staining solution on the coverslip for 20 minutes at room temperature. To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.

   Note: Phalloidin conjugates also can be included with fluorescently-labeled antibodies in blocking buffer during the secondary antibody incubation step in your regular immunofluorescence staining protocol.
8. Wash 2-3 times with PBS.
9. For biotin phalloidin, continue with biotin detection using labeled streptavidin or anti-biotin antibody. For fluorescent phalloidins, proceed to imaging.
10. Alexa Fluor dye phalloidins are photostable enough to image in PBS, but for best results we recommend mounting with antifade mounting medium.

    Note: Alexa Fluor 647, and Alexa Fluor 680 are cyanine-based dyes and are not compatible with VECTASHIELD mounting media Immunological antifade mounting media are compatible with a wide range of fluorescent dyes, including cyanine dyes and Alexa Fluor dyes.

    Note: Fluorescent dyes can affect the stability of phalloidin staining. For best results, store phalloidin-stained samples at 4^{o}C, protected from light, and image within 24 hours. Staining with phalloidins conjugated to rhodamine-based

    Alexa Fluor (488, 546, 568, 594, 633, is stable for up to a week after staining when specimens are stored at 4^{o}C, protected from light.

Staining Living Cells

Fluorescently-labeled phalloidin is not cell-permeant and has therefore has not been used extensively with living cells. However, living cells have been labeled by pinocytosis or unknown mechanism. In general, a larger amount of stain will be needed for staining living cells. Alternatively, fluorescent phalloidins have also been injected into cells for monitoring actin distribution and cell motility .