Revision 5

Alexa Fluor® 647 Phalloidin

Store at -20C

#8940

300 units

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:
IF-F, IF-IC	All

Description

Alexa Fluor® 647 Phalloidin allows researchers to fluorescently stain the cytoskeleton through the binding of phalloidin to F-actin. This product is intended for use on fixed and permeabilized samples due to the toxicity associated with phalloidin. After reconstitution the stock solution provides enough material to perform 200 assays based on a 1:100 dilution and a 100 µl assay volume.

Alexa Fluor® 647 Fluorescent Properties: Excitation: 650, Emission: 668.

Background

Actin, a ubiquitous eukaryotic protein, is the major component of the cytoskeleton. At least six isoforms are known in mammals. Nonmuscle β- and γ-actin, also known as cytoplasmic actin, are predominantly expressed in nonmuscle cells, controlling cell structure and motility (1). Actin exists mainly as a fibrous polymer, F-actin. In response to cytoskeletal reorganizing signals during processes such as cytokinesis, endocytosis, or stress, cofilin promotes fragmentation and depolymerization of F-actin, resulting in an increase in the monomeric globular form, G-actin (2). Phalloidin is a natually occuring toxic bicyclic peptide found in the deathcap toadstool, Amanita phalloides, that rapidly binds to F-actin with strong affinity (3).

Storage

This material is provided as lyophilized solid that is stable for 1 year at -20°C, desiccated and protected from light. Phalloidin conjugates should be reconstituted in anhydrous DMSO to make stock solutions, please refer to the directions for use for details. Once reconstituted in DMSO, stock solutions are stable for 1 year at -20°C. Stability in aqueous solutions is low and the conjugate should only be in the presence of an aqueous solution during incubation with cells.

Directions for Use

To make a 100X stock, reconstitute the lyophilized material in 200 µl anhydrous DMSO. Fix cells for 15 minutes using fresh, methanol-free 4% formaldehyde, then rinse three times in PBS for 5 minutes each. Once fixed, please follow CST protocol for immunostaining. Following incubation of the primary and secondary antibodies, Alexa Fluor® 647 Phalloidin can be diluted 1:100 (1 µl per 100 µl assay volume) in PBS and added to cells. Allow to incubate for 15 minutes at room temperature, then rinse once with PBS. Coverslip slides with ProLong® Gold antifade reagent and examine specimen using appropriate excitation wavelength.

Note: Product usage information for this product has changed effective September 01 2024. Previous recommendations were to use either methanol or DMSO as a solvent for lyophilization of the material; It is now recommended to ONLY use DMSO.

Background References

1. Herman, I.M. (1993) *Curr Opin Cell Biol* 5, 48-55.
2. Condeelis, J. (2001) *Trends Cell Biol* 11, 288-93.
3. Lengsfeld, A.M. et al. (1974) *Proc Natl Acad Sci U S A* 71, 2803-7.

Applications Key

IF-F: Immunofluorescence (Frozen) **IF-IC:** Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

All: All Species Expected

Trademarks and Patents

Limited Uses

force or effect.

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