Revision 4

Store at -20C

#12848

DyLight 350 Phalloidin

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

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

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

Description

DyLight 350 Phalloidin allows researchers to fluorescently label the cytoskeleton through the binding of phalloidin to F-actin. This product is not intended for use on live cells due to the toxicity associated with phalloidin. After reconstitution the stock solution provides enough material to perform 50 assays based on a 1:10 dilution and a 100 µl assay volume.

DyLight 350 Fluorescent Properties: Excitation: 356nm, Emission: 423nm.

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 pure methanol to make stock solutions, please refer to the directions for use for details. Once reconstituted in pure methanol, stock solutions are stable for 1 year at -20°C, desiccated and protected from light. 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 10X stock, reconstitute the lyophilized material in 0.5 ml pure methanol.

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 Cell Signaling Technology protocol for immunostaining. Following incubation of the primary and secondary antibodies, DyLight 350 Phalloidin can be diluted 1:10 (10 µl per 100 µl assay volume) in PBS and added to the cells. Allow to incubate for 15 minutes at room temperature, then rinse once with PBS. Coverslip slides with ProLong Gold Antifade Reagent #9071 and examine specimen using appropriate excitation wavelength.

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

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