Store at -20°C

#12935

DyLight 488 Phalloidin

Cell Signaling TECHNOLOGY®

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

Applications	Species Cross-Reactivity
IF-IC	All

Description: DyLight 488 Phalloidin allows researchers to fluorescently label the cytoskeleton of fixed cells 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 3000 assays based on a 1:400 dilution and a 100 µl assay volume.

DyLight 488 Fluorescent Properties: Excitation: 495 nm, Emission: 521 nm.

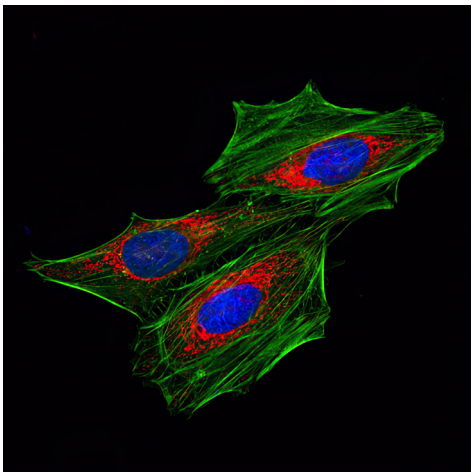
Background: Actin is a ubiquitous eukaryotic protein and a major component of the cytoskeleton. At least six isoforms are known in mammals. Nonmuscle β- and γ-actin, also known as cytoplasmic actin, are predominantly expressed in nonmuscle cells to control cell structure and motility (1). Actin exists mainly as a fibrous polymer called 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 G-actin form (2). Phalloidin is a naturally occurring toxic bicyclic peptide found in the deathcap toadstool, *Amanita phalloides*, which rapidly binds to F-actin with strong affinity (3).

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 USA* 71, 2803-7.



Confocal immunofluorescent analysis of HeLa cells using Cox IV (4D11-B3-E8) Mouse mAb #11967 (red). Actin filaments were labeled with DyLight 488 Phalloidin (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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 400X stock, reconstitute the lyophilized material in 750 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 Cell Signaling Technology protocol for immunofluorescence. Following incubation of the primary and secondary antibodies, DyLight 488 Phalloidin can be diluted 1:400 (0.25 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.

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.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: H—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.