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DyLight™ 350 Phalloidin

Cell Signaling TECHNOLOGY®

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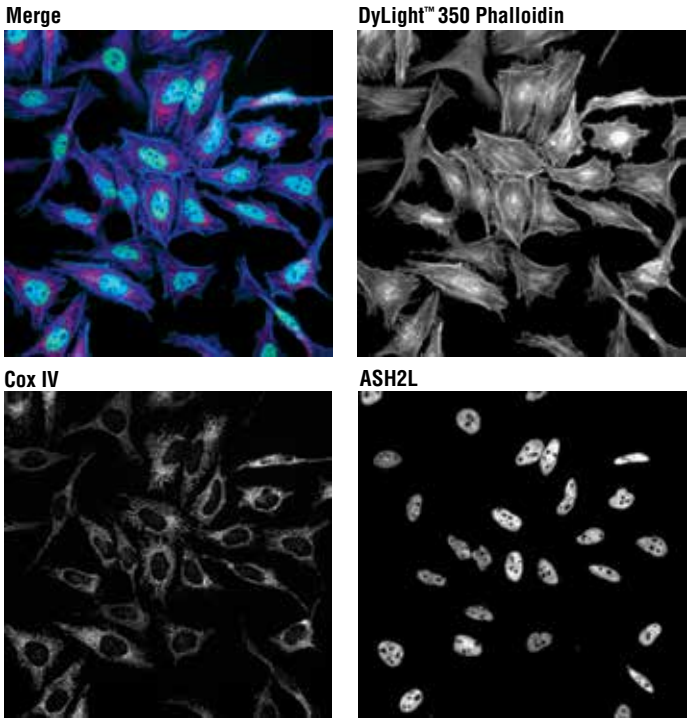
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity
IF-IC, IF-F	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: 356 nm, Emission: 423 nm.

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).



Immunofluorescent analysis of HeLa cells using Cox IV (4D11-B3-E8) Mouse mAb #11967 (red) and ASH2L (D93F6) XP® Rabbit mAb #5019 (green). Actin filaments were labeled with DyLight™ 350 Phalloidin (blue).

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 CST 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 USA* 71, 2803-7.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **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.