

DyLight™ 650 Phalloidin



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

Applications
IF-IC, IF-F

Species Cross-Reactivity
All

Description: DyLight™ 650 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 300 assays based on a 1:20 dilution and a 100 µl assay volume.

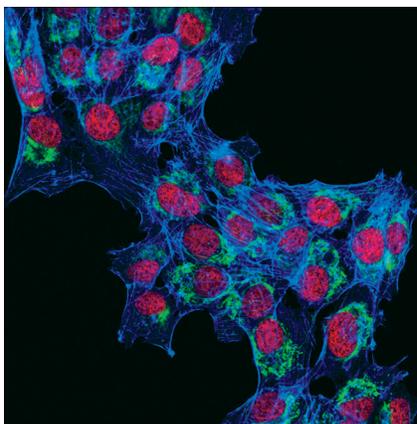
DyLight™ 650 Fluorescent Properties: Excitation: 651 nm, Emission: 672 nm.

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.

HeLa



Confocal immunofluorescent analysis of HeLa cells using Cox IV (4D11-B3-E8) Mouse mAb #11967 (green). Actin filaments were labeled with DyLight™ 650 phalloidin (blue pseudocolor). Red = Propidium Iodide (PI)/RNase Staining Solution #4087.

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 20X stock, reconstitute the lyophilized material in 1.5 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 immunofluorescence. Following incubation of the primary and secondary antibodies, DyLight™ 650 Phalloidin can be diluted 1:20 (5 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.

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

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