

Phalloidin, DyLight 633

Catalog Number21840

Product data sheet

Details		Species Reactivity	
Size	300 units	Species reactivity	Many
Host/Isotope	Not Applicable	Tested Applications	
Type	Control	Immunohistochemistry (IHC)	Assay-dependent
Conjugate	DyLight® 633	Immunocytochemistry (ICC/IF)	1-5 units/mL
Form	Lyophilized	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Concentration	300 U/mL		
Storage Conditions	-20°C		

Product specific information

Format: 300 units of lyophilized DyLight 633-Phalloidin (300 units/mL) in methanol. Prepare stock solution by adding 1000 µL of pure methanol to the vial and gently mix. 21840 has been successfully used in immunofluorescence and immunohistochemistry. DyLight 633-Phalloidin has an excitation /emission of 636/658 nm and molecular weight of 1506.77 g/mole. DyLight 633-Phalloidin Stock Solution is prepared in methanol, and is also soluble in DMF, or DMSO. The unused Stock Solution should be promptly stored at -20°C in a foil pouch with desiccant, and protected from light. The stock solutions are stable for at least one year. Staining cells with some of the DyLight Phalloidin conjugates may require the use of a higher concentration of the phalloidin conjugate; therefore to minimize the amount of methanol added to the cells, the vial contents could be dissolved in 0.5 mL methanol to yield a final concentration of 600units/mL. Working solution of 1unit/mL (per 96-well plate) can be prepared by diluting 20 µL of the Stock Solution in 5.98 mL of PBS and mixing well. Typical staining procedure adds 50 µL of Working Solution (i.e. 1 to 5 unit/mL) to a each well. Incubate cells in the dark for 30 minutes at room temperature (optimal staining times varies from 10 minutes to 3 hours depending on cell type). Aspirate and wash cells three times in PBS after incubation. If desired, probe with specific primary antibodies followed by secondary antibodies conjugated to any compatible fluorophores before using DyLight 633-Phalloidin conjugate.

Background/Target Information

Phalloidin is a bicyclic peptide that belongs to a family of toxins isolated from the deadly Amanita phalloides “death cap” mushroom and is commonly used as a counterstain (similar to DAPI or Hoechst) in cell biology and histology imaging applications to selectively label F-actin in fixed cells, permeabilized cells, and cell-free experiments. Labeled phalloidin conjugates have similar affinity for both large and small filaments and bind in a stoichiometric ratio of about one phallotoxin per actin subunit in both muscle and non-muscle cells. Phalloidins reportedly do not bind to monomeric G-actin, unlike some antibodies against actin. The dynamics of the actin polymerization in cells are important for a variety of cellular processes from cell motility to cell shape, from muscular contraction to cytokinesis, and more.

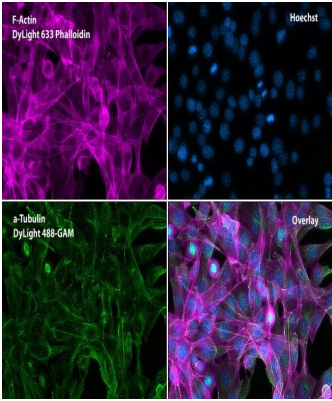
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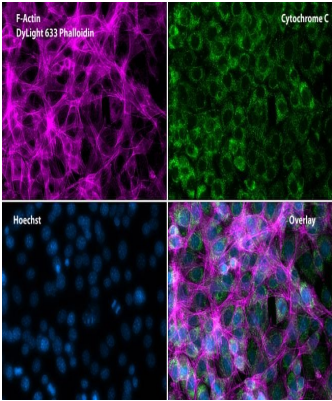
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Product Images For Phalloidin, DyLight 633



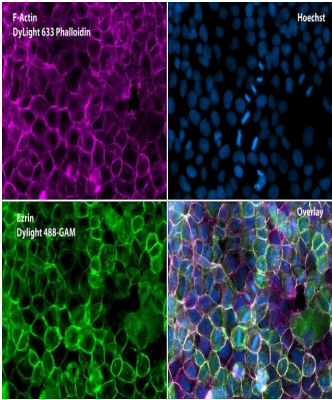
Phalloidin Control (21840) in ICC/IF

Immunofluorescent analysis of Phalloidin (magenta) and alpha-Tubulin (green) in NIH 3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes at room temperature and blocked with 2% BSA (Product # 37525) in PBS + 0.1% Triton X-100 for 30 minutes at room temperature. Cells were probed with an alpha-Tubulin monoclonal antibody (Product # MA1-19162) at a dilution of 1:1000 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:250 for 30 minutes at room temperature. Actin was stained with DyLight 633 Phalloidin (Product # 21840) at a dilution of 1:120 (2.5units/mL final concentration) and nuclei (blue) were stained with Hoechst (Product # 62249) at a concentration of 1 µg/mL for 30 minutes. Images were taken on a Zeiss Axio Observer Z1 microscope at 20X magnification.



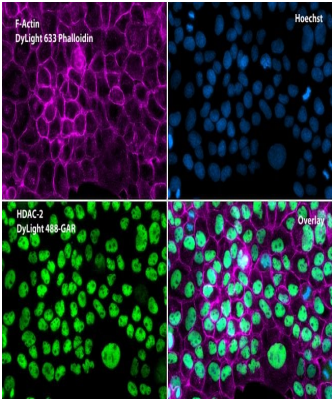
Phalloidin Control (21840) in ICC/IF

Immunofluorescent analysis of Phalloidin (magenta) and Cytochrome c (green) in NIH 3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes at room temperature and blocked with 2% BSA (Product # 37525) in PBS + 0.1% Triton X-100 for 30 minutes at room temperature. Cells were probed with a Cytochrome c monoclonal antibody (Product # MA5-11823) at a dilution of 1:75 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:250 for 30 minutes at room temperature. Actin was stained with DyLight 633 Phalloidin (Product # 21840) at a dilution of 1:120 (2.5units/mL final concentration) and nuclei (blue) were stained with Hoechst (Product # 62249) at a concentration of 1 µg/mL for 30 minutes. Images were taken on a Zeiss Axio Observer Z1 microscope at 20X magnification.



Phalloidin Control (21840) in ICC/IF

Immunofluorescent analysis of Phalloidin (magenta) and Ezrin (green) in A431 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes at room temperature and blocked with 2% BSA (Product # 37525) in PBS + 0.1% Triton X-100 for 30 minutes at room temperature. Cells were probed with an Ezrin monoclonal antibody (Product # MA5-13862) at a dilution of 1:75 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:250 for 30 minutes at room temperature. Actin was stained with DyLight 633 Phalloidin (Product # 21840) at a dilution of 1:120 (2.5units/mL final concentration) and nuclei (blue) were stained with Hoechst (Product # 62249) at a concentration of 1 µg/mL for 30 minutes. Images were taken on a Zeiss Axio Observer Z1 microscope at 20X magnification.



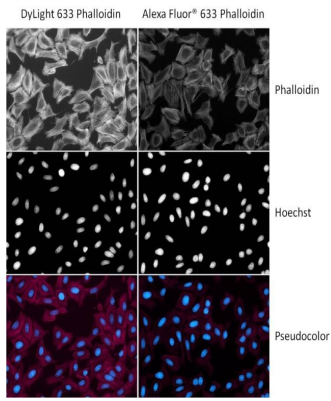
Phalloidin Control (21840) in ICC/IF

Immunofluorescent analysis of Phalloidin (magenta) and HDAC2 (green) in A431 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes at room temperature and blocked with 2% BSA (Product # 37525) in PBS + 0.1% Triton X-100 for 30 minutes at room temperature. Cells were probed with an HDAC2 polyclonal antibody (Product # PA1-861) at a dilution of 1:75 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-rabbit IgG secondary antibody (Product # 35552) at a dilution of 1:250 for 30 minutes at room temperature. Actin was stained with DyLight 633 Phalloidin (Product # 21840) at a dilution of 1:120 (2.5units/mL final concentration) and nuclei (blue) were stained with Hoechst (Product # 62249) at a concentration of 1 µg/mL for 30 minutes. Images were taken on a Zeiss Axio Observer Z1 microscope at 20X magnification.

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Phalloidin Control (21840) in ICC/IF

Immunofluorescent analysis of Phalloidin in U2OS cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 2% BSA in PBS (Product # 37525) containing 0.1% Triton X-100 for 30 minutes at room temperature. Cells were probed with DyLight 633 Phalloidin (Product # 21840) or Alexa Fluor® 633 Phalloidin, each diluted to a final concentration of 2.5 units/mL in PBS, for 30 minutes. Nuclei were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Zeiss Axio Observer Z1 microscope with a 20X objective.

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