

PRODUCT INFORMATION

Phalloidin-iFluor™ 532 Conjugate

Item No. 20551

Purity: ≥90%
Supplied as: A solution in DMSO (1000X)
Storage: -20°C
Stability: 2 years
Special Conditions: Light sensitive

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

Phalloidin-iFluor™ 532 Conjugate is a fluorescein-based dye used to label actin filaments (F-actin). Phalloidin binds to F-actin and prevents depolymerization.¹ It also inhibits the ATP hydrolysis action of F-actin.² Phalloidin-iFluor™ 532 can be used to label tissue, cells, and cell-free preparations that have been formaldehyde-fixed and permeabilized. It displays excitation/emission spectra of 532/556 nm, respectively. Phalloidin-iFluor™ 532 can be used at nanomolar concentrations in conjunction with additional dyes for multi-labeling purposes.

Assay Protocol

NOTE: Warm the vial to room temperature and centrifuge briefly before opening.

1. Prepare a 1X phalloidin conjugate working solution: Add 1 µl of the supplied stock solution (1,000X phalloidin conjugate in DMSO) to 1 ml of PBS containing 1% BSA.

NOTE 1: The unused 1,000X DMSO stock solution of phalloidin conjugate should be aliquoted and stored at -20°C protected from light.

NOTE 2: Different cell types might stain differently. The concentration of phalloidin conjugate working solution should be prepared accordingly.

2. Stain the cells:
 - a. Perform formaldehyde fixation by incubating the cells with 3-4% formaldehyde in PBS at room temperature for 10-30 minutes.
NOTE: Avoid any methanol-containing fixatives because methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.
 - b. Rinse the fixed cells 2-3 times in PBS.
 - c. *Optional:* Add 0.1% Triton X-100 in PBS to fixed cells (from Step 2b) for 3-5 minutes to increase permeability. Rinse the cells 2-3 times in PBS.
 - d. Add 100 µl of 1X phalloidin conjugate working solution (from Step 1) per well of a 96-well plate into the fixed cells (from Step 2b or 2c) and stain the cells at room temperature for 20-90 minutes.
 - e. Rinse cells gently with PBS 2-3 times to remove excess phalloidin conjugate before plating, sealing, and imaging under microscope.

References

1. Lengsfeld, A.M., Löw, I., Wieland, T., *et al.* Interaction of phalloidin with actin. *Proc. Natl. Acad. Sci. USA* **71**(7), 2803-2807 (1974).
2. Löw, I., Dancker, P., and Wieland, T. Stabilization of F-actin by phalloidin. Reversal of the destabilizing effect of cytochalasin B. *FEBS Lett.* **54**(2), 263-265 (1975).

WARNING

THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

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