PRODUCT INFORMATION

Phalloidin-iFluor™ 488 Conjugate

Item No. 20549

Purity: ≥90%
Supplied as: A solution in DMSO
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™ 488 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™ 488 can be used to label tissue, cells, and cell-free preparations that have been formaldehyde-fixed and permeabilized. It displays excitation/emission spectra of 493/517 nm, respectively. Phalloidin-iFluor™ 488 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 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).

CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.CAYMANCHEM.COM