Phalloidin-TRITC

Cat #: orb2719400 (manual)

Size: 50T, 300T

1. Introduction

Phalloidin is a bicyclic peptide toxin isolated from the death cap mushroom, Amanita phalloides. Fluorescent phalloidins bind F-actin with nanomolar affinity and are water-soluble. Phalloidin conjugates are convenient probes for labeling, identifying, and quantifying actin filaments in animal or plant samples, including formaldehyde-fixed, permeabilized tissue sections, cell cultures, and cell-free experiments. They are widely used for qualitative and quantitative analysis of F-actin.

2. Contents

Phalloidin-TRITC

3. Storage

Store at -20°C and protect from light.

4. Operating Instructions

4.1 50T:

(1) Stock preparation: Prepare 1000x stock solution by dissolving lyophilized phalloidin (50T) in 5uL DMSO. This stock solution can be sub-packaged then stored at -20 °C. Solutions should be prepared fresh and protected from light whenever possible.

(2) Working solution: Prepare 1x working solution by adding 1 µL of Phalloidin stock solution to 1 mL of PBS with 1% BSA.

4.2 300T:

(1) Stock preparation: Prepare 1000x stock solution by dissolving lyophilized phalloidin (300T) in 30uL DMSO. This stock solution can be sub-packaged then stored at -20 °C. Solutions should be prepared fresh and protected from light whenever possible.

(2) Working solution: Prepare 1x working solution by adding 1 µL of Phalloidin stock solution to 1 mL of PBS with 1% BSA.

Note: Different cell types might be stained differently. The concentration of phalloidin conjugate working solution should be prepared accordingly.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

5. Assay Procedure

Staining fixed cells

The following protocol describes the staining procedure for adherent cells.

(1) Wash cells 3 times with PBS.

(2) Fix cells on ice with 4% formaldehyde solution in PBS for 10-30 minutes.

Note: Methanol can disrupt actin during the fixation process. Therefore, it is best to avoid any methanol containing fixatives or other solvent-based fixatives. The preferred fixative is methanol-free formaldehyde.

(3) Wash cells 3 times with PBS.

(4) Permeabilize cells with 0.5% Triton X-100 in PBS at room temperature for 10 minutes.

(5) Wash cells 3 times with PBS.

(6) Add 100 µL/well (96-well plate) phalloidin-conjugate working solution. Place the staining solution on the coverslip for 20-90 minutes at room temperature.

Note: Staining volume can be adjusted according to the sample. To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.

(7) Wash 2-3 times with PBS.

(8) Image using fluorescence microscopy. Fluorescent phalloidins are photostable enough to image in PBS, but for best results we recommend mounting with antifade mounting medium.

Precautions

Always wear lab coats, gloves and goggles when working with our products although they are low-risk chemicals.

For research use only. Not for diagnostic or therapeutic procedures.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558