Protocol for Phalloidin-TRITC

This is intended as a guide only - optimization may be needed.

In Brief

Phalloidin is a phallotoxin isolated from *Amanita phalloides*, the death cap mushroom. Fluorescent phallotoxins stain F-actin at nanomolar concentrations. They are water soluble and produce low non-specific binding.

Reconstitution

Dissolve the vial contents in 1.5 mL methanol or DMSO. This will yield a solution with a final concentration of approximately 7.3 µM.

This stock solution can be further diluted as required for the intended application, we recommend a dilution of approximately 40X.

Storage Condition

Stock solutions can be frozen for one year when stored at ≤–20°C, desiccated, and protected from light.

Formaldehyde-Fixed Cells

- 2X Wash cells with warmed phosphate-buffered saline (PBS), pH 7.4.
- Use 3.7% formaldehyde solution (in PBS) to fix cells. Leave for 10 minutes at room temperature (recommend using methanol-free formaldehyde).
- 2X Wash cells with PBS.
- Place each coverslip in a glass petri dish and extract it with a solution of acetone at ≤–20°C or 0.1% Triton X-100 in PBS. Leave for ~ 5 minutes.
- 2X Wash cells with PBS.
- When staining, dilute 5 µL Phalloidin-FITC methanolic stock solution into 200 µL PBS for each coverslip. To reduce nonspecific background staining add 1% bovine serum albumin (BSA). Place inside container and eave for 20 minutes.
- 2X Wash cells with PBS.

Fixation, Permeabilization, and Phallotoxin-FITC Staining

Prepare a 1 mL solution containing 50 to 100 µg/mL lysopalmitoylphosphatidylcholine and 3.7% formaldehyde, add approximately 50 µL of Phalloidin-FITC methanolic stock solution. Apply staining solution to cells and leave for 20 minutes at 4°C. 3X Wash cells with PBS. View coverslip.

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Live cells

Phallotoxins are usually not cell-permeable, but it is possible to label some types of cells. Pinocytosis, microinjections and unknown entry methods by hepatocytes have all been documented. Please conduct a literature search to find an appropriate protocol for your experiments.