

## Protocol for Phalloidin-FITC

*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 6.7  $\mu$ M.

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  $\leq -20^{\circ}\text{C}$ , desiccated, and protected from light.

### Formaldehyde-Fixed Cells

1. 2X Wash cells with warmed phosphate-buffered saline (PBS), pH 7.4.
2. Use 3.7% formaldehyde solution (in PBS) to fix cells. Leave for 10 minutes at room temperature (recommend using methanol-free formaldehyde).
3. 2X Wash cells with PBS.
4. Place each coverslip in a glass petri dish and extract it with a solution of acetone at  $\leq -20^{\circ}\text{C}$  or 0.1% Triton X-100 in PBS. Leave for ~ 5 minutes.
5. 2X Wash cells with PBS.
6. When staining, dilute 5  $\mu$ L Phalloidin-FITC methanolic stock solution into 200  $\mu$ L PBS for each coverslip. To reduce nonspecific background staining add 1% bovine serum albumin (BSA). Place inside container and leave for 20 minutes.
7. 2X Wash cells with PBS.

### Fixation, Permeabilization, and Phalloidin-FITC Staining

Prepare a 1 mL solution containing 50 to 100  $\mu$ g/mL lysopalmitoylphosphatidylcholine and 3.7% formaldehyde, approximately 50  $\mu$ L of Phalloidin-FITC methanolic stock solution. Apply staining solution to cells and leave for 20 minutes at  $4^{\circ}\text{C}$ . 3X Wash cells with PBS. View coverslip.

### Live cells

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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.