Cat.	Product Name	Ex (nm)	Em (nm)
C4332	Phalloidin-AMCA Conjugate	350	450
C4340	Phalloidin-Fluorescein Conjugate	492	518
C4233	Phalloidin-Tetramethylrhodamine Conjugate	546	575
B8325	Phalloidin-California Red Conjugate	583	605

Protocol

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

Prepare 1X Phalloidin conjugate working solution: by adding 1 µL of 1000X Phalloidin conjugate solution to 1 mL of PBS with 1% BSA.

Note

a. The unused 1000X DMSO stock solution of phalloidin conjugate should be aliquoted and stored at -20℃ and protected from light.

b. Different cell types might be stained differently. The concentration of phalloidin conjugate working solution might need to be adjusted.

2. Stain the cells:

2.1 Remove culture medium and wash the cells for 2-3 min.

Perform formaldehyde fixation. Incubate cells with 3.0–4.0 % formaldehyde in PBS at room temperature for 10–30 minutes.

Note: Avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.

2.2 Rinse the fixed cells 2–3 times in PBS under room temperature.

2.3 Add 0.1% Triton X-100 in PBS into fixed cells (from Step 2.2) for 3 to 5 minutes to increase permeability.

Rinse the cells 2–3 times in PBS.

2.4 Add 100 µL/well (96-well plate) of phalloidin conjugate working solution (from Step 1) into the fixed cells (from Step 2.3), and stain the cells at room temperature for 20 to 90 minutes.

For coverslips in 24-well plate, use 200 µL/well to cover the cells or you can adjust to an appropriate volume according to your samples.

2.5 Rinse cells gently with PBS 2 to 3 times to remove excess phalloidin conjugate before plating, sealing and imaging under microscope.