

## AMCA Phalloidin(Blue)

**Cat:** CA1690

**Size:** 300T

**Storage:** -15°C, avoid light.

**Properties:** liquid (DMSO dissolved products, no need to dissolve)

### Product description:

AMCA phalloidin selectively bind to F-actin. Used in nanomolar concentrations, porcinocyclic peptide derivatives are convenient probes for labeling, identifying, and quantifying F-actin in formaldehyde-fixed and permeated tissue sections, cell cultures, or cell-free experiments. Actin is a globular, approximately 42kDa protein that is present in almost all eukaryotic cells. It is also one of the highly conserved proteins, diverging no more than 20% from algae and different species of humans. Actin is a monomer subunit of two filaments in cells: microfilaments, one of the three main components of the cytoskeleton, and microfilaments, which are part of the contractile apparatus in muscle cells. As such, actin is involved in many important cellular processes, including muscle contraction, cell movement, cell division and cytoplasmic division, vesicle and organelle movement, cell signaling, and the establishment and maintenance of cell connections and cell shape.

Phalloidin bind to actin filaments more tightly than actin monomers, resulting in a reduced rate constant of actin subunits dissociating from filament-like ends, essentially stabilizing the actin filaments by preventing filament depolymerization. In addition, phalloidin was found to inhibit the ATP-hydrolyzing activity of F-actin. Phalloidin act differently in cells at different concentrations. When introduced into the cytoplasm at low concentrations, porcinocyclic peptide aggregates less polymerized forms of cytoplasmic actin as well as filamentins into a stable "island" of aggregated actin polymers, but it does not interfere with the stressed fibers, the thick bundles of microfilaments. The properties of porcinocyclic peptides are a useful tool for studying the distribution of F-actin in cells by labeling porcinocyclic peptides with fluorescent analogues and staining actin filaments with them for light microscopy. Fluorescent derivatives of phalloidin have proven to be very useful in locating actin filaments in living or fixed cells, as well as in visualizing individual actin filaments in vitro. Fluorescent phalloidin derivatives have been used as an important tool in high-resolution actin studies.

- Ex/Em (nm) =346/434nm

### Protocols:

1. Add 1μL of the AMCA phalloidin conjugate solution to 1mL of PBS containing 1% BSA.

Note 1: The unused reserve solution of the phalloidin conjugate should be equally divided and stored at -20°C, away from light.

Note 2: Different cell types may stain differently. The concentration of the working solution of the phalloidin conjugate should be prepared accordingly.

2. Staining cells:

2.1 Perform formaldehyde fixation. Incubate cells containing 3.0-4.0% formaldehyde in PBS for 10-30 minutes at room temperature.

Note: Avoid using any fixatives containing methanol, as methanol can destroy actin during the fixation process. The preferred fixative is formaldehyde, which does not contain methanol.

2.2 Flush the fixed cells with PBS 2-3 times.

2.3 Optional: Add 0.1% Triton X-100 to PBS to fix cells for 3 to 5 minutes to increase permeability. Rinse the cells with PBS 2 to 3 times.

2.4 Add 100 $\mu$ L/well (96-well plate) phalloidin conjugated working solution to the stationary cells and stain the cells at room temperature for 20 to 90 minutes.

2.5 The cells are gently rinsed with PBS 2-3 times to remove excess phalloidin conjugates before the addition of a cover glass, which is then performed under a microscope, sealed and imaged.

**Note:**

1. Prepare the sample in the microporous plate hole
2. Remove the liquid from the sample in the plate
3. Add AMCA phalloidin solution (100 $\mu$ L/ well)
4. Stain the cells at room temperature for 20 to 90 minutes
5. Clean the cells to examine the sample under a microscope

Note: Heat the vial to room temperature and centrifuge briefly before opening.

**Apply:**

Labeling, identifying, and quantifying formaldehyde fixed and permeated F-actin in tissue sections, cell cultures, or cell-free experiments.