

# Phalloidin dye conjugates

## 1 Contents

Cat. No.	Product Name	Ex (nm)	Em (nm)	Size	Package
HY-K0901	AMCA Phalloidin	344	432	300 T	30 µL
HY-K0902	Fluorescein Phalloidin	497	516	300 T	30 µL
HY-K0903	Rhodamine Phalloidin	552	578	300 T	30 µL

## 2 General Information

Phalloidin is a mushroom-derived toxin which can be used to specifically label F-actins (actin filaments) of the cytoskeleton. It binds to all variants of actin filaments in many different species of animals and plants. Typically, Phalloidin is used conjugated to a fluorescent dye. Phalloidin binds F-actins with high selectivity while fluorescent dye provides stable and bright fluorescence.

MCE offers three Phalloidin dye conjugates, AMCA Phalloidin (HY-K0901, blue fluorescence), Fluorescein Phalloidin (HY-K0902, green fluorescence) and Rhodamine Phalloidin (HY-K0903, orange fluorescence). These Phalloidin dye conjugates are 1000× stock solution in DMSO which is convenient for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments.

## 3 Protocol

1. Prepare 1× Phalloidin dye conjugate working solution:

Add 1 µL of 1000× Phalloidin dye conjugate DMSO solution to 1 mL of PBS containing with 1% BSA.

Note 1: Different cell types might be stained differently. The concentration of Phalloidin dye conjugate working solution should be prepared accordingly.

Note 2: 1% bovine serum albumin (BSA) is used to reduce nonspecific background staining.

2. Stain the cells:

2.1 Wash cells 2–3 times with PBS. Fix the cells in 3.7% methanol-free formaldehyde solution in PBS for 10-30 minutes at room temperature.

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

2.2 Wash the fixed cells 2–3 times in PBS.

2.3 Optional: Add 0.1% Triton X-100 in PBS into fixed cells for 3–5 minutes to increase permeability. Wash the cells 2–3 times in PBS.

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

2.5 Rinse cells gently with PBS 2–3 times to remove excess Phalloidin dye conjugate.

2.6 Run fluorescence microscope or other equipments at desired Ex/Em wavelengths (AMCA Phalloidin, Ex/Em=344/432 nm; Fluorescein Phalloidin, Ex/Em=497/516nm; Rhodamine Phalloidin, Ex/Em=552/578 nm).

## 4 Storage

The solutions should be stable for at least 6 months if store at -20°C.

Protect the Phalloidin dye conjugates from light, and avoid freeze/thaw cycles.

## 5 Precautions

1. One Test(T) of Phalloidin dye conjugates is equivalent to 0.1  $\mu\text{L}$  of the DMSO stock solution (100  $\mu\text{L}$  working solution).
2. Phalloidin is toxic although the amount of toxin present in a vial could be lethal only to a mosquito ( $\text{LD}_{50}$  of phalloidin = 2 mg/kg). Please handle with care.
3. This product is for R&D use only, not for drug, household, or other uses.