



## TraKine™ F-actin Staining Kit (Orange Fluorescence)

Cat #: KTC4009

Size: 50 T/300 T/1000 T

	<b>F-actin Staining Kit (Orange Fluorescence)</b>		
<b>REF</b>	Cat #: KTC4009	<b>LOT</b>	Lot #: Refer to product label
	<b>Fluorescence excitation/emission:</b> 593 nm/614 nm		
	<b>Storage:</b> Stored at -20°C for 6 months		

### Assay Principle

Phalloidin belongs to a class of toxins called phallotoxins, which are isolated from the death cap mushroom (*Amanita phalloides*). It is a bicyclic peptide that binds to F-actin specifically. Therefore, the distribution of F-actin can be very conveniently studied by using a fluorescent dye-labeled phalloidin. Inside the phalloidin, there is an unusual thioether bridge between cysteine and tryptophan, which can form an inner ring structure. When the pH is raised, the thioether is cleaved and the phalloidin loses its affinity for actin. TraKine™ F-actin Staining Kit (Orange Fluorescence) uses a orange fluorescent (Ex/Em=593 nm/614 nm) phalloidin conjugate selectively bound to F-actins, it is much higher photostability than the fluorescein-phalloidin conjugates. Phalloidin derivatives are convenient probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, which can stain F-actins in cell cultures or cell-free experiments at nanomolar levels.

### Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	50 T	300 T	1000 T	
Phalloidin (Orange Fluorescence) (200×)	25 µL	150 µL	500 µL	-20°C, protected from light
Assay Buffer (5×)	1 mL	6 mL	20 mL	4°C

### Materials Required but Not Supplied

- Fluorescence Microscope
- 96-well plate (cell culture)
- Pipettes and pipette tips
- Deionized water
- Phosphate buffered saline (PBS) (pH 7.4)
- 4% paraformaldehyde (PBS preparation)
- 0.1% Triton X-100 (PBS preparation)

## Reagent Preparation

**Phalloidin (Orange Fluorescence) (200×):** Before use, warm to room temperature. The remaining working solution can be stored at -20°C after aliquoting to avoid repeated freezing and thawing.

**Assay Buffer (5×):** Before use, dilute to 1×Assay Buffer with deionized water, and then heat to 37°C. Store at 4°C.

**Staining Solution:** Add 5 µL Phalloidin (Orange Fluorescence) (200×) to 1 mL 1×Assay Buffer, and increase the volume according to the number of experiments.

## Assay Procedure

**Note:** As the optimal staining conditions may vary among different cell types, we recommend that a suitable concentration of Phalloidin (Orange Fluorescence) be determined.

1. Grow cells directly on a coverslip in 96 well dish. Incubate in a CO<sub>2</sub> incubator at 37°C for at least 24 h before treatment.
2. Wash cells with PBS twice.
3. Perform cells with ice-cold 4% formaldehyde fixation in PBS for 15-30 min on the ice.

**Note:** Methanol can damage actin during the fixation process. So, it is best to avoid fixatives containing any methanol. The preferred fixative is formaldehyde free of methanol.

4. Wash cells with PBS three times.
5. Cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min at room temperature.
6. Wash cells with PBS three times.
7. Add 100 µL/well (96-well plate) of staining solution into cells, and stain cells in the dark for 30 min at room temperature.
8. Wash cells with PBS twice.
9. Cells were observed under a fluorescence microscope. The orange fluorescence dye-labeled phalloidin has good light stability and the sample can be imaged in PBS, but for best effect, it can be observed using an anti-fluorescence quencher.

**Note:** The orange fluorescence dye-labeled phalloidin does not have cell permeability, therefore, it is not widely used for living cell markers. However, it has been reported that living cells may be marked by pinocytosis or by unknown mechanisms. In general, more dye is needed to stain living cells. Alternatively, the orange fluorescence dye-labeled phalloidin can also be injected into cells for monitoring actin distribution and cell movement.

## Recommended Products

Catalog No.	Product Name
KTC4001	TraKine™ Cell Plasma Membrane Staining Kit (Green Fluorescence)
KTC4002	TraKine™ Cell Plasma Membrane Staining Kit (Orange Fluorescence)
KTC4003	TraKine™ Mitochondrion Staining Kit (Green Fluorescence)
KTC4004	TraKine™ Mitochondrion Staining Kit (Orange Fluorescence)
KTC4005	TraKine™ Mitochondrion and Nuclear Staining Kit
KTC4008	TraKine™ F-actin Staining Kit (Green Fluorescence)

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.