AbFluor™ 488-Phalloidin

Cat #: BMD0082 Size: 500 T

	AbFluor™ 488-Phalloidin		
REF	**Cat #:** BMD0082	LOT	**Lot #:** Refer to product label
	Application range: To label cellular F-actin		**Excitation/Emission wavelengths:** Ex/Em=490/515 nm
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Phalloidin has the molecular formula $C_{35}H_{48}N_8O_{11}S$, molecular weight 788.87 and CAS number 17466-45-4. 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. AbFluor™ 488-Phalloidin selectively bound to F-actin, it is much higher photostability than the fluorescein-phalloidin conjugates. Phalloidin derivatives are convenient probes for labeling, identifying and quantitating F-actin in formaldehyde-fixed and permeabilized tissue sections, which can stain F-actin in cell cultures or cell-free experiments at nanomolar levels.

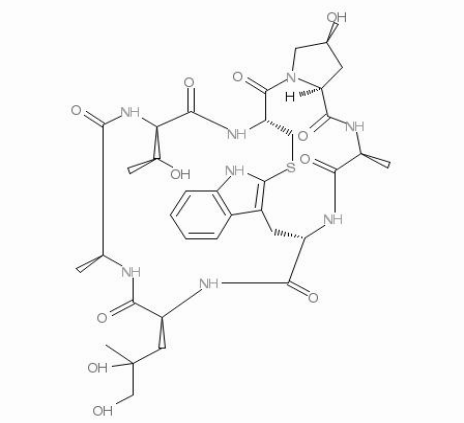

Figure1. Molecular diagram

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
AbFluor™ 488-Phalloidin	1	-20℃, protected from light

Materials Required but Not Supplied

- Fluorescent microscope, precision pipettes, disposable pipette tips, 96-well plate for cell culture
- PBS, DMSO

Assay Procedure

1. Prepare 100×AbFluor™ 488-Phalloidin DMSO stock solution: by adding 500 μL of DMSO into the powder form vials. Then should be aliquoted and stored at -20℃. Protected from light and avoid freeze/thaw cycles.
2. Prepare 1×AbFluor™ 488-Phalloidin conjugate working solution: by adding 1 μL of 100× Phalloidin conjugate DMSO solution to 99 μL of 1× PBS.

Note: Because the optimal staining concentration used for different cells may be different, the dilution ratio can be appropriately adjusted according to the actual staining effect.

3. Grow cells directly on a coverslip in 96 well dish. Incubate in a CO_2 incubator at 37℃ for at least 24 h before treatment.
4. Wash cells with PBS twice before fix cells with ice-cold 4% formaldehyde fixation 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.

5. Wash cells with PBS three times and cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min at room temperature.
6. Wash cells with PBS three times and Add 100 μL/well (96-well plate) of AbFluor™ 488-Phalloidin staining solution into cells, and stain cells for 30 min at room temperature.
7. Wash cells with PBS twice and viewed and recorded using fluorescence microscope. The AbFluor™ 488-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.

Precautions

1. Please immediately centrifugal the product to the bottom of the tube before use, and then conduct the subsequent experiments.
2. Phalloidin is harmful to human body, please wear a laboratory coat and disposable gloves to operate, pay attention to appropriate protection.
3. Fluorescent dyes all have quenching problems, please try to avoid light to slow down the quenching.

Disclaimer

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