AbFluor™ 488-Phalloidin

Item NO.	Product Name
BMD0082	AbFluor™ 488-Phalloidin

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 2018010

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INTRODUCTION

Background & 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.

AbFluor™ 488-Phalloidin 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.

Storage/Stability

Stable for at least 12 months if store at -20°C. Protect this product from light. Gel pack with blue ice.

Assay Restrictions

- This product is intended for research use only. Not for use in diagnostic procedures.
- Phalloidin is toxic, it should be handled with care.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Cat.#	Product Name	Unit	Ex (nm)	Em (nm)	Storage conditions
BMD0082	AbFluor™ 488-Phalloidin	500T	490	515	-20°C, Protect from light

Other supplies required, Not Supplied

- Pipettes and pipette tips
- Fluorescence Microscopy
- 96-well plate for cell culture
- Phosphate-buffered saline (PBS)

Technical hints

- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Ensure this product is at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Note: As the optimal staining conditions may vary among different cell types, we recommend that a suitable concentration of AbFluor™ 488-Phalloidin should be determined.

Reagent Preparation

Prepare 100× Phalloidin DMSO stock solution: by adding 500 µl of DMSO into the powder form vials. Then should be aliquoted and stored at -20°C. Protected from light and avoid freeze/thaw cycles.

Prepare 1× Phalloidin conjugate working solution: by adding 1 µl of 100× Phalloidin conjugate DMSO solution to 100 µl of 1× PBS.

Recommended procedures

Detection by Fluorescence Microscopy

1. Formaldehyde-Fixed Cells.

1.1. Grow cells directly on a coverslip in 96 well dish. Incubate in a CO_2 incubator at 37°C for at least 24 hours before treatment.

1.2. Wash cells with PBS twice in pH 7.4 (PBS).

1.3. Fix cells with ice-cold 4% formaldehyde fixation in PBS for 15-30 minutes 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.

2. Stain cells.

2.1. Wash cells (from Step 1.3) with PBS three times.

2.2. Cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min at room temperature.

2.3. Wash cells with PBS three times.

2.4. Add 100 µL/well (96-well plate) of AbFluor™ 488-Phalloidin staining solution into cells, and stain cells for 30 minutes at room temperature.

2.5. Wash cells with PBS twice.

2.6. Cells were observed under a 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.