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Vari Fluor 555-Phalloidin

Cat. No.: HY-D1816

Target: Fluorescent Dye

Pathway: Others

Storage: -20°C, protect from light
* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)

BIOLOGICAL ACTIVITY

Description

Vari Fluor 555-Phalloidin is a fluorescent derivative of Phalloidin that specifically labels myofilament proteins and exhibits red fluorescence when labeled, allowing for fluorescence imaging using the PE channel (Ex/Em=550 nm/561 nm)[1].

In Vitro

General Introdction
Ghost pen cyclic peptide is a toxin isolated from the deadly umbrella-shaped mushrooms. It is a bicyclic peptide that binds specifically to F-actin. Therefore, the distribution of F-actin can be easily studied by labeling the peptide with a fluorescent dye. Inside the cyclic peptide, an uncommon thioether bridge between cysteine and tryptophan forms an inner ring structure. At elevated pH, the thioether is cleaved and the peptide loses its affinity for actin.
Vari Fluor-Phalloidin is a fluorescent derivative of the VF series of dye-labeled phalloidin that specifically labels actin. The fluorescence is highly stable and can be retained in the cell for more than a week.
One unit (T) of VF-Phalloidin refers to the amount of dye used to stain a slide loaded with cells.

General Protocol
1.Preparation of VF-Phalloidin Working Solution
1.1 Preparation of storage solution
Take appropriate amount of methanol or sterile water to dissolve the lyophilized powder in the brown tube to configure the mother liquor.
Add 0.25 mL of dissolution solution to 50T dye.
Add 1.5 mL of solvent to 300T size dye.
Note: VF-Phalloidin storage solution is recommended to be stored at -20 ⊠ or -80 ⊠ after dispensing and protected from light, and methanol is recommended for long-term storage.
1.2 Preparation of working solution
Dilute the stock solution with pre-warmed serum-free cell culture medium or PBS at 1:40-1:200, i.e., dilute 1-5 μL of VF-Phalloidin stock solution per 200 μL of PBS.
Note: Please adjust the concentration of VF-Phalloidin working solution according to the actual situation and use it as it is.
2. Cell staining (adherent cells)
2.1 Culture adherent cells on sterile coverslips and wash them 3 times with PBS.
2.2 Fix the cells with PBS solution containing 4% formaldehyde for 20 min at room temperature.
Note: Methanol can destroy actin during fixation. Therefore, it is best to avoid fixatives containing any methanol. The preferred fixative is methanol-free formaldehyde.
2.3 Wash the cells 3 times with PBS.
2.4 The cells are permeabilized with 0.4% Triton X-100 in PBS for 10 min at room temperature.
2.5 Wash the cells three times with PBS.

2.6 Add 200 μL of dye working solution, shake gently to completely cover the cells, and incubate at room temperature away from light for 15-45 minutes.
Note: The chromosome volume can be adjusted according to the sample. To avoid evaporation of the dye solution during incubation, place the coverslip in a sealed container.
2.7 Pipette off the dye working solution and wash 2-3 times with PBS or culture medium.
2.8 Use fluorescence microscope or flow cytometer to observe under the corresponding channel.

Precautions
1.Fluorescently-labeled Ghost Pen Cyclic Peptides are not cell permeable and therefore have not been widely used for live cell labeling. However, it has been reported that living cells may be labeled by cytosolic drinking or by unknown mechanisms. Generally, more dye is required to stain living cells. Alternatively, fluorescently labeled ghost pen cyclic peptides can be injected into cells to monitor actin distribution and cell movement.
2.The actual content of the dye is small, so dissolve it directly in the tube upon receipt and perform the experiment.
3.Please centrifuge the product instantly to the bottom of the tube before use.
4.This product is limited to scientific research use by professionals, and is not to be used for clinical diagnosis or treatment, food or medicine.
5.For your safety and health, please wear lab coat and disposable gloves.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Borovikov YuS, et al. The effect of phallotoxins on the structure of F-actin in myosin-free ghost muscle fibres of rabbit. FEBS Lett. 1984 Oct 29;176(2):441-3.

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