TRITC Phalloidin

Cat : CA1610
Size: 300T
Storage: -20℃ dry storage away from light, valid for 1 year.
Product description:

Ghost pen cyclic peptide and its derivatives can stain F-actin at nanomolar concentration, which is a very practical and convenient probe, usually used for specific fluorescence staining of F-actin in tissue sections or cell cultures. In addition, F-actin derivatives are small, with a diameter of 12-15A and a molecular weight of < 2000 Daltons. The labeled F-ActiN still maintains many of its pre-labeled functions. For example, actin binding proteins such as myosin, tropomyosin, DNase I, etc., can still react; Filaments labeled with phopinocyclic peptide can still penetrate the solid myosin matrix; And the muscle fibers extracted by glycerol can still contract after labeling.

The binding of Phalloidin prevents the dissociation of filamentous actin (microfilaments), stabilizes the structure of microfilaments, and thus disrupts the polymerization-depolymerizing homeostasis of microfilaments. This property reduces the critical concentration (CC) at which actin polymerization occurs to < 1μg/mL and, therefore, can be used as a polymerization promoter. In addition, porcinocyclic peptide can inhibit the ATP-hydrolyzing activity of F-actin.

This product is TRITC (tetramethylRhodamine isothiocyanate) labeled ghost ring peptide, strong staining reaction specificity, high contrast, has better staining effect than Actin antibody, suitable for qualitative and quantitative determination of F-actin. In addition, F-actin combined with this product can still maintain many biological characteristics of actin itself. Moreover, the combination of this product has no species difference and has wide applicability.

Product properties:

CAS#	915013-10-4
Molecular Formula	$C_{60}H_{70}N_{12}O_{13}S_2$
Molecular Weight (Molecular Weight)	1231.4
Maximum excitation/emission wavelength (Ex/Em)	540~546/565~575nm
Polypeptide Sequence (Sequence)	TRITC-bicyclic(Ala-DThr-Cys-cis-4-hydroxy-Pro-Ala-2-mercapto-Trp-4-hydroxy-5-amino-Leu)(S-3 to 6)
(Appearance)	Purple liquid

Materials:

1. (Optional) methanol
2. 1×PBS buffer, pH 7.4, cell culture grade
3. Fixed solution 4% paraformaldehyde (dissolved in PBS buffer)
4. Acetone or permeating solution 0.5% Triton X-100 (dissolved in PBS buffer)

5. Fluoromount-G water-soluble sealers (DAPI free), DAPI
6. (Optional) DAPI Fluoromount-G Water-soluble sealer (DAPI free)
7. (Optional) BSA, standard grade
8. Slide and cover slide
9. Seal liquid around cover glass (such as clear nail polish)
10. Fluorescent microscope or confocal microscope with TRITC excitation/emission filter and DAPI excitation/emission filter.

Protocols:

1. Prepare the working fluid

This product is supplied as a 20μM storage solution dissolved in methanol. It is recommended that after receiving the product, according to the single usage, the mother liquor should be subpackaged in a small amount, frozen at -20℃ away from light, and stable for one year. Before starting the experiment, use 1×PBS buffer to dilute the storage solution to the required working concentration. Recommended working concentration: 80 to 100nM. Ready to use.

2. Dye steps

1）Cell creep grow >24h, so that its density reaches 50-60% confluent degree.
2）The culture medium was sucked off and the cells were washed twice at 37℃ preheated 1×PBS (pH 7.4).
3）The cells were fixed with 4% formaldehyde solution dissolved in PBS and fixed at room temperature for 10-30min.

Note: Avoid the fixative containing methanol components, as methanol may destroy actin during the immobilization process.

4）At room temperature, wash the cells with PBS 2 to 3 times for 10min each time.
5）At room temperature, the cells were dehydrated with acetone (≤-20 ° C) or permeated with 0.5% Triton X-100 solution for 5min.
6）At room temperature, the cells were washed with PBS 2 to 3 times for 10min each time.
7）Take 100μl/ well (96-well plate) prepared TRITC-labeled ghost pen cyclic peptide working solution, cover the cells on the cover glass, and incubate at room temperature for 30min away from light (usually incubation at 4℃~37℃ can be used).

Note: In order to reduce the background, 1% BSA can be added to the TRITC-labeled ghost pen cyclic peptide working solution; In addition, to avoid evaporation of the solution during incubation, transfer the cover glass to an airtight container.

8）Clean the cover glass with PBS 3 times for 5min each time.
9）Restain the nucleus with 100μl/ well (96-well plate) readyuse DAPI solution (concentration: 100nm) for about 30s.
10) Clean the cover glass with PBS, then invert onto a slide already dripping with a drop of Fluoromount-G water-soluble sealer. Gently sassafras the excess sealer off using a paper towel, then

11) seal the sealer permanently with nail polish. The specimen slides prepared by this method can be stored at 4 ° C away from light and can usually be continued for F-actin staining analysis within 6 months.
Note: It is also possible to simplify the procedure by directly using an anti-fluorescence quenched seal containing DAPI combined with steps 9) 10).
11) For fluorescence observation under fluorescence microscope or confocal microscope, select TRITC excitation/emission filter (Ex/Em=540/570nm) and DAPI excitation/emission filter (Ex/Em=364/454nm).
Note:
1. The definition of a unit (T) of fluorescent labeled ghost pen cyclic peptide: according to the recommended working liquid concentration of 200nM, each dosage of 100μL dyeing working liquid, can detect 300 times; According to the working liquid concentration of 100nM, each amount of 200μL dyeing working liquid, the number of detections can also be 300 times.
2. Ghost pen cyclic peptide is toxic, need to be careful operation.
3. The product is limited to professionals for life science research, shall not be used for clinical diagnosis or treatment, shall not be used for food or medicine, shall not be stored in ordinary residential.
4. The product must be operated by qualified professional and technical personnel while wearing a mask/gloves/lab coat and comply with the biological laboratory safety operating procedures!

Related literature:

[1] Xiaojiao Sun, Dechao Zhao, Fangping Lu, et al. Hydrogen Sulfide Regulates Muscle RING Finger-1 Protein S- Sulfhyd -ration at Cys44 to Prevent Cardiac Structural Damage in Diabetic Cardiomyopathy. British Journal of Pharmacology banner. February 2019. (IF 6.810)

[2] Fan Wu, Jingqi Zheng,Zhixiong Li, et al. Halloysite nanotubes coated 3D printed PLA pattern for guiding human mesenchymal stem cells (hMSCs) orientation. Chemical Engineering Journal. March 2019. (IF 8.355)

Note: For more information about this product, please refer to the Solarbio website.