

Product Data Sheet

Product Name: Fluo-5N AM

Cat. No.: GC26364

Chemical Properties

Cas. No.

Formula $C_{50}H_{47}F_2N_3O_{25}$

M.Wt 1127.92

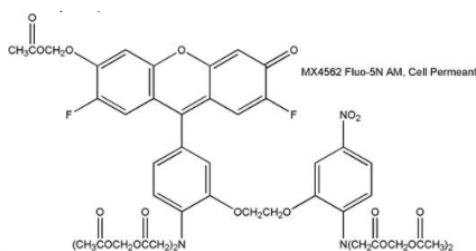
Solubility 5.64mg/mL in DMSO

Storage -20°C, Sealed storage, away from moisture and light.

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Usage

A. Reagent preparation

1) Preparation of PluronicF-127 mother liquor: Weigh 100mg of PluronicF-127 powder and add 500 to it μ Prepare 20% (w/v) DMSO mother liquor. The dissolution process requires heating at 40-50 °C for 20-30 minutes. Store the solution at room temperature without refrigeration. If there is crystal precipitation, it can be dissolved after reheating without affecting its use.

2) HHBS Buffer (1X Hank's Balanced Salt Solution with 20mM HEPES buffer, pH 7.2) or other physiological buffer B, operating steps

1) Dissolve Fluo-5NAM in anhydrous DMSO to prepare a 2-5mM storage solution, or take the pre prepared Fluo-5NAM storage solution out of room temperature for reheating. (For example, if it is prepared into a 4mM mother liquor, it needs to be diluted to 50%) μ Add 11ul anhydrous DMSO to

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Fluo-5NA M. Before preparing the Fluo-5NAM working solution, it is sometimes necessary to add an appropriate amount of 20% Pluronic F-127 solution to the Fluo-5NAM storage solution to enhance the water solubility of the AM probe.

[Note ①]: Before preparing the Fluo-5N AM staining solution, add a certain volume of 20% Pluronic F-127 solution to the Fluo-5NAM+DMSO storage solution, so that the final working concentration of Pluronic F-127 is about 0.02%.

2) Dilute Fluo-5NAM+DMSO storage solution to 1-20 using HHBS or other physiological buffer (containing calcium and magnesium) μ M's working fluid.

[Note ①]: The recommended loading concentration for Fluo-5N AM application in most cells is 4-5 μ M. The specific usage concentration needs to be optimized according to experimental requirements. To avoid cytotoxicity caused by overloading, it is recommended to use the lowest probe concentration as much as possible on the basis of obtaining effective results.

3) [Optional] If intracellular (such as CHO cells) contain organic anion transporters, Probenecid (1-2.5mM) or Sulfapyrazone (0.1-0.5mM) may need to be added to the cell culture medium to reduce the leakage level of the de esterification probe.

4) Add the prepared Fluo-5NAM staining solution to the cells in the amount required to cover the cells. Incubate at room temperature or 37 °C for 20-120 minutes.

[Note ①]: If serum containing culture medium is used, serum esterase will degrade AM, thereby reducing the loading effect of Fluo-5NAM. However, phenol red culture medium may slightly increase the background value. It is recommended to wash the cells 2-3 times before adding staining solution.

5) Suck off the staining solution and wash the cells 1-2 times with HHBS or other physiological buffer (if necessary, use buffer containing transporter inhibitors such as 1mM probenecid) to remove residual probes.

6) Incubate at room temperature for another 30 minutes to ensure complete de esterification of intracellular AM.

7) Use appropriate excitation/emission wavelengths to detect fluorescence

matters needing attention

1) Fluorescent dyes all have quenching problems, please try to avoid light as much as possible to slow down fluorescence quenching.

2) Acetoxymethyl ester (AM) is prone to moisture absorption. After taking it out of the refrigerator, please place it in a dry environment at room temperature before opening it. Due to the trace amount of the reagent, please centrifuge it briefly before opening to ensure that the powder falls into the bottom of the tube.

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- 3) Fluo-5NAM will solidify and stick to the bottom, wall, or cover of the centrifuge tube at lower temperatures such as 4 °C or ice bath. It can be incubated at 20-25 °C for a while until fully melted before use.
- 4) For the first use of Fluo-5NAM, it is recommended to prepare and use the storage solution immediately, divide it into individual doses, and strictly seal, dry, and freeze it at ≤ -20 °C to prevent moisture. To ensure good experimental results, try to use it in a short period of time.
- 5) For your safety and health, please wear laboratory clothes and disposable gloves when operating.

Background

Fluo-5N is a structural analogue of Fluo-4, with lower calcium binding affinity, making it highly suitable for calcium levels at 1 μ M. Detection of intracellular calcium ions within the M~1mM range, while the calcium ion reactions of Fluo-3 and Fluo-4 reach saturation within this range. Fluo-5N is compatible with the 488nm excitation of argon ion lasers, making it suitable for confocal microscopy, flow cytometry, fluorescence enzyme-linked immunosorbent assay (ELISA), and other instruments. The excitation and emission wavelengths are 494 and 516 nm, respectively. Once combined with calcium ions, the fluorescence intensity increases by more than 100 times and wavelength migration is very minimal.

Fluo-5NAM is an acetyl methyl ester derivative of Fluo-5N, which has cell membrane permeability and can easily enter cells with simple cultivation.

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