

# Product Information

## Fluo-3, AM ester

**Catalog Number:** F3005-1mg, F3015-50 µL

**Packaging Size:** 1 mg, 50 µL (2 mM)

## Product Description

**Color & Form & Solubility:** Orange red solid soluble in DMSO

**CAS number:** 121714-22-5

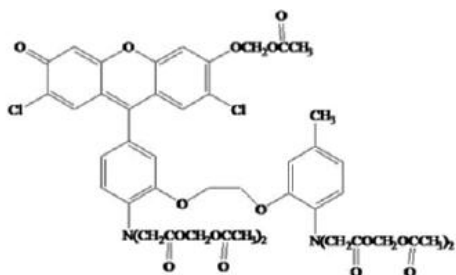
**Shelf life:** 12 months

$\lambda$  Ex/ $\lambda$  Em (after hydrolysis) = 506 nm/526 nm (low or high [Ca<sup>2+</sup> ])

**Molecular formula:** C<sub>51</sub>H<sub>50</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>23</sub>

**Molecular weight:** approx. 1129.9

**Molecular Structural Formula:**



## Storage

Store desiccated at -20°C and protect from light, especially when in solution.

## Description

Fluo-3, AM ester is a fluorescent dye that can penetrate the cell membrane. Fluo-3, AM ester can be cut into Fluo-3 by endogenous esterase after entering the cell, and then remain in the cell. Fluo-3 can bind with calcium ions, which can produce strong fluorescence. The maximum excitation wavelength is 506 nm and the maximum emission wavelength is 526 nm.

## Application Protocols

1. Fluo-3,AM was dissolved in anhydrous DMSO to prepare 2-5 mM storage solution. Fluo-3,AM of solution form should be taken out to come to room temperature.

2. Fluo-3, AM solution was diluted by HBSS to prepare 4 µM Fluo-3, AM working fluid.

**Note:** In order to avoid the cytotoxicity caused by overloading, it is suggested using the minimum probe concentration as far as possible on the basis of effective results.

(optional) If the effect of Fluo-3 entering cells is not good, add 20% Pluronic F127 solution to Fluo-3, AM/DMSO solution to prevent Fluo-3,AM from aggregating in HBSS and promoting the entry into cells. The final concentration of Pluronic F127 is controlled at 0.04-0.05%.

**Note:** ① Preparation of Pluronic F-127 liquor: 100 mg Pluronic F-127 was added into 0.5 mL DMSO to prepare 20% (w/v) DMSO mother liquor. The dissolution process needs to be heated at 40-50 °C for 20-30 min. Store solution at room temperature, do not refrigerate. If crystallization occurs, it can be reheated and dissolved without affecting its use.

② Pluronic F127 can reduce the stability of Fluo-3,AM. Therefore, it is only recommended to add in the working fluid in the preparation, but not recommended to add it to the storage liquid for long-term storage.

4. Take out the pre-cultured cells, remove the medium and wash the cells three times with PBS or HBSS solution.

5. Fluo-3,AM working fluid were added to the cells and cultured at 37°C for 10-60 min.



**Note:** If the incubation temperature and time can not be determined in the first experiment, it is suggested to try incubation at 37°C for 20 minutes to observe the fluorescence effect. If the cell death is more, the time or temperature should be shortened; if the fluorescence intensity is too weak, the time should be prolonged.

6. Remove Fluo-3, AM working fluid. Cells were washed three times with PBS or HBSS and then re-suspended with PBS or HBSS to form a solution of  $1 \times 10^5$  cells/mL.

7. Incubation at 37 °C for 10 minutes to ensure complete deesterification of AM bodies in the cells.

8. Calcium ion fluorescence detection (excitation wavelength 506 nm, emission wavelength 526 nm).

### Notes

1. If the serum containing medium is used, the serum esterase will decompose AM body, thus reducing the effect of

Fluo-3,AM entering cells. In addition, the medium containing phenol red will make the background value slightly higher, so before processing the working fluid, we should try to remove the residue of the medium.

2. Quenching of fluorescent dyes is a problem. Please avoid light as much as possible to slow down the quenching of fluorescent dyes.

3. Fluo-3,AM is easy to absorb moisture. After removing from the refrigerator, please make sure that it is kept at room temperature in a dry environment before opening. Because the reagent is very small, please centrifuge it briefly before opening to ensure that the powder falls to the bottom of the pipe.

4. Fluo-3,AM is easy to decompose when it meets water. If it can't be used up at one time, it is suggested to separate and store the liquid in small quantities.

