

Fura-2 AM

Cat: IF1510

Storage: Powder: -20°C, 2 years; Insolvent (mother liquid): -20°C, 6 months; -80°C, 1 year (protect from light)

Introduction

Fura-2 AM is a cell-permeable, high-affinity fluorescent probe for calcium ions. Fura-2 AM is the ester form of Fura-2, a polar, acidic compound that is unable to enter living cells, and the addition of acetoxymethyl ester (AM) to its negative moiety significantly increases cell permeability.

After Fura-2 AM penetrates the cell membrane and enters living cells, it is de-esterified by intracellular esterases into Fura-2 free acid, which binds specifically to intracytoplasmic free Ca^{2+} (binding ratio of 1:1). When Fura-2 binds to free Ca^{2+} in the cytoplasm, it exhibits an absorption shift, with the peak excitation wavelength changing from 380 nm to 340 nm, while the peak emission near 510 nm remains unchanged. A quantitative relationship exists between fluorescence intensity and the concentration of bound Ca^{2+} . Fura-2 is generally excited at 340 nm (Ca^{2+} -Fura-2) and 380 nm (Fura-2), and the intracellular Ca^{2+} concentration is calculated by using the ratio of fluorescence intensities corresponding to the two excitations. Ratio measurements minimize the effects of photobleaching in the mixture, leakage of the fluorescent probe, inhomogeneous loading of the probe, and differences in cell thickness, thus providing more reliable and reproducible results.

Parameter

CAS: 108964-32-5

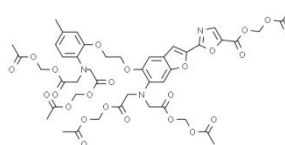
Molecular Formula: $\text{C}_{44}\text{H}_{47}\text{N}_3\text{O}_{24}$

Molecular Weight: 1001.85

Purity: $\geq 95\%$

Appearance: Solid

Solubility: Soluble in DMSO



Protocols (only for reference)

Preparation of storage solution

Prepare a 1 mM stock solution in DMSO. For example, 50 μg of Fura-2 AM powder is dissolved in 49.9 μL of DMSO.

Note:

- Unused storage solution is recommended to be stored in portions at -20°C to avoid repeated freezing and thawing.
- Moisture-absorbing DMSO has a significant effect on the solubility of the product, use freshly opened DMSO.

Preparation of working fluid

Dilute the reservoir solution with a suitable buffer (e.g. HBSS, etc.) to formulate 1-10 μM of Fura-2

AM working fluid.

Note:

- a. The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems.
- b. If it is found to be difficult to dissolve, it can be sonicated appropriately to promote dissolution.
- c. Please adjust the concentration of the working fluid according to the actual situation, and use it now.

Loading probe

1. Remove the precultured cells, remove the medium and wash the cells 3 times with HBSS solution. If serum-containing medium is used, esterases in the serum will break down the AM bodies, thus decreasing the entry of Fura-2 AM into the cells. Additionally, medium containing phenol red will result in a slightly higher background value, so try to remove as much of the medium as possible before adding the working solution.
2. Add Fura 2-AM working solution in an amount that covers the cells.
3. Incubate the cells at 37°C for 10-60 min to remove the Fura-2 AM working solution. Regarding the incubation time, if you are not sure for the first time, it is recommended to incubate the cells for 30 min to see the fluorescence effect: if the cells are dying more, shorten the time; if the fluorescence intensity is too weak, lengthen the time.
4. Wash the cells 3 times with HBSS solution to fully remove the residual Fura-2 AM working solution. Then add HBSS solution to cover the cells.
5. Incubate in the incubator at 37°C for about 20-30 minutes to ensure complete de-esterification of AM bodies in the cells. If intracellular esterase activity is low, it is recommended to strictly follow this procedure; this step can be ignored for experiments with cells with high esterase activity.
6. Detect cells by flow cytometry or other equipment with excitation wavelengths of 380 nm (Fura 2) and 340 nm (Calcium-Fura 2) and emission wavelength of 510 nm.

Calculation formula

Intracellular free Ca^{2+} concentration = $K_d (F_0/F_s) (R - R_{\min}) / (R_{\max} - R)$

*The K_d value in the equation is 224 nmol/l, F_0 and F_s represent the fluorescence intensity measured at zero Ca^{2+} and saturation, respectively, R is the experimentally observed fluorescence ratio, and R_{\max} and R_{\min} are the maximum and minimum fluorescence ratios, respectively.

*Labeling conditions vary depending on the cell type, so please determine the optimal conditions before each experiment. The above method is for reference only.

Note

1. If the effect of Fura-2 AM into the cell is not good, you can use Pluronic F-127, the latter can prevent the polymerization of Fura-2 AM in the buffer and can promote its entry into the cell. Pluronic F-127 is first dissolved in DMSO to a concentration of 20% (W/V), and then added directly into the working solution of Fura-2 AM according to the experimental needs to the

final concentration of 0.04-0.05% (this concentration is for reference only, please adjust according to the specific experimental requirements). concentration of 0.04-0.05% (this concentration is for reference only, please adjust according to the specific experimental requirements).

2. Fura-2 AM dry powder is easy to absorb moisture, after taking it out of the refrigerator, please make sure it is put in a dry environment to room temperature before opening. Due to the extremely small amount of this product, before opening, please flick the wall of the tube several times or centrifuge it instantly to ensure that the powder falls to the bottom of the tube.
3. It is recommended to find the optimal experimental conditions by figuring out the amount of cells, the final concentration of calcium fluorescent probe, the incubation time, etc. before the formal experiment.
4. All fluorescent dyes have extraction problems, please avoid light to slow down the fluorescence extraction.
5. For your safety and health, please wear lab coat and disposable gloves.
6. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in a residential area.

Related Products

<i>P6791</i>	<i>Pluronic F-127 (20% in DMSO)</i>
<i>IF0150</i>	<i>Fluo-3AM</i>
<i>IF1500</i>	<i>Fluo-4AM</i>
<i>IKA1012-1</i>	<i>Calcium ion concentration Detection Kit (Rhod-2 AM)</i>