

Product Data Sheet

Product Name: Fura-2 AM

Cat. No.: GC16578

Chemical Properties

Cas. No. 108964-32-5

Chemical Name bis(acetoxymethyl) 2,2'-((2-(5-((acetoxymethoxy)carbonyl)oxazol-2-yl)-4-(2-(2-(bis(2-(acetoxymethoxy)-2-oxoethyl)amino)-5-methylphenoxy)ethoxy)benzofuran-5-yl)azanediyl)d iacetate

SMILES O=C(CN(CC(OCOC(C)=O)=O)C1=CC=C2OC(C3=NC=C(C(OCOC(C)=O)=O)O3)=CC2=C1OCCOC4=CC(C)=CC=C4N(CC(OCOC(C)=O)=O)CC(OCOC(C)=O)=O)OCOC(C)=O

Formula $C_{44}H_{47}N_3O_{24}$

M.Wt 1001.85

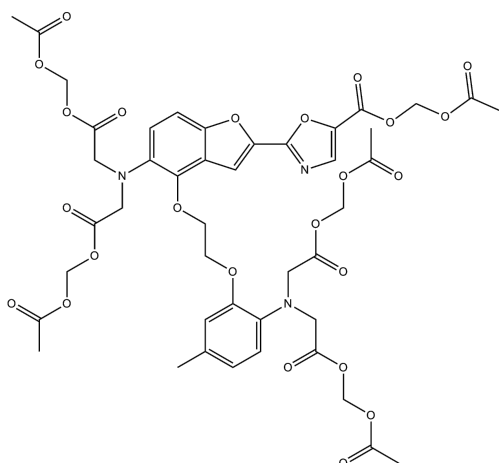
Solubility DMSO: 10 mg/ml

Storage Store at -20°C, protect from light, unstable in solution, ready to use.

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

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Evaluation of calcium content using Fura-2 AM

This protocol only provides a guideline, and should be modified according to your specific needs

1. Prepare dyeing solution

(1) Prepare stock solution: Prepare Fura-2 AM stock solution with a concentration of 1-10mM in high-quality anhydrous DMSO. Notice: Aliquot the unused stock solution and store it in the dark at -20°C or -80°C to avoid repeated freezing and thawing.

(2) Prepare working solution: Dilute the stock solution with a suitable buffer (such as serum- and phenol red-free culture medium or PBS) to prepare a working solution with a concentration of 1-10µM.

Notice:

1) When preparing the Fura-2 AM working solution, it is sometimes necessary to add an appropriate amount of 20% Pluronic F-127 solution to the stock solution to enhance the water solubility of the Fura-2 AM;

2) Pluronic F-127 can prevent Fura-2 AM from aggregating in solution and promote better entry of the probe into cells. However, Pluronic F-127 can reduce the stability of the Fura-2 AM, so it is only recommended to be added when preparing the working solution. It is not recommended to add the stock solution for long-term storage;

3) Prepare 20% (w/v) Pluronic F-127 stock solution: weigh 100mg Pluronic F-127 powder, add 500µl DMSO, heat at 40-50°C for 20-30min, and store at room temperature. If crystals precipitate, they can be reheated to dissolve without affecting use;

4) (Optional) GLPBIO provides dissolved Pluronic F-127 (20% Solution in DMSO), product number: GB30091;

5) Add an equal volume of 20% Pluronic F-127 solution to the stock solution so that the final working concentration of Pluronic F-127 is approximately 0.02%;

6) Please adjust the concentration of the working fluid according to the actual situation, and prepare it now to avoid repeated freezing and thawing.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

2. Cell suspension staining

(1) Suspension cells: centrifuge at 4°C and 1000g for 3-5 minutes, discard the supernatant, and wash twice with PBS or other buffer, 5 minutes each time;

(2) Adherent cells: wash twice with PBS or other buffers, add trypsin to digest the cells, and centrifuge at 1000g for 3-5 minutes after digestion is completed;

(3) Add Fura-2 AM working solution to resuspend the cells, and incubate at 37°C or room temperature in the dark for 20min-2h. The optimal incubation time for different cells is different, please explore according to your specific experimental needs.

Note:

1) The recommended working concentration of Fura-2 AM in most cells is 1-5 μ M, and the specific concentration needs to be optimized according to experimental requirements. In order to avoid cytotoxicity caused by overloading, use the lowest probe concentration possible based on achieving effective results;

2) (Optional) If the cells contain organic anion transporters, you may need to add probenecid (1-2.5mM) or sulfinpyrazone (0.1-0.25mM) to the cell culture medium. to reduce the leakage level of the deesterification probe. The stock solution of probenecid or sulfinpyrazone is alkaline, so the pH needs to be readjusted after adding the culture medium;

3) If serum-containing medium is used, serum lactonase will degrade Fura-2 AM, thereby reducing the dye loading effect; while phenol red-containing medium will make the background value slightly higher. Wash the cells 2 to 3 times before adding the staining working solution;

4) Lowering the probe loading temperature may reduce the compartmentalization of the probe.

(4) After the incubation, centrifuge at 1000g for 5 minutes to remove the staining solution, add PBS, and wash 2-3 times to remove residual probes;

(5) Incubate at room temperature for another 30 minutes to ensure complete deesterification of intracellular Fura-2 AM.

3. Cell adhesion staining

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

- (1) Culture adherent cells on sterile coverslips;
- (2) Remove the coverslip from the culture medium, suck out the excess culture medium, and place the coverslip in a humid environment;
- (3) Add 100µl of Fura-2 AM working solution from one corner of the coverslip and shake gently to evenly cover all cells with the dye;
- (4) Incubate in the dark at 37°C or room temperature for 20min-2h. The optimal incubation time for different cells is different, please explore according to your specific experimental needs;
- (5) After the incubation, discard the dye working solution and wash the coverslip 2 to 3 times with PBS;
- (6) Incubate at room temperature for 30 minutes.

4. Precautions:

- (1) Fluorescent dyes all have quenching problems. Please avoid light as much as possible to slow down fluorescence quenching.
- (2) For your safety and health, please wear a lab coat and disposable gloves when operating
- (3) Fura-2 AM should be stored in the dark throughout the experiment.
- (4) This protocol and all reagents are for scientific research use only.

References:

[1] Barreto-Chang O L, Dolmetsch R E. Calcium imaging of cortical neurons using Fura-2 AM[J]. Journal of visualized experiments: JoVE, 2009 (23): 1067.

Background

Fura-2 AM, an esterified cell-permeant form of the Ca^{2+} indicator fura-2, is useful for measuring calcium signals [1]. Fura-2 AM will be de-esterified by intracellular esterases, thereby being trapped within the cell and enabling Fura-2 to bind to calcium ions [2]. Fura-2 AM has been widely used to measure the content of cytoplasmic free Ca^{2+} in monolayers and suspensions of various types of

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

animal cells^[3]. Fura-2 AM can be utilized to develop new detection tools for measuring the concentration of free calcium ions within bacteria (such as *Selenomonas ruminantium*)^[4]. Fura-2 AM can be used in calcium imaging systems to analyze the increase in calcium ion concentration in cortical neurons^[5].

References:

- [1] Oakes S G, Martin II W J, Lisek C A, et al. Incomplete hydrolysis of the calcium indicator precursor fura-2 pentaacetoxymethyl ester (fura-2 AM) by cells[J]. Analytical biochemistry, 1988, 169(1): 159-166.
- [2] Gulaboski R, Pereira C M, Cordeiro M N D S, et al. Redox properties of the calcium chelator Fura-2 in mimetic biomembranes[J]. Cell calcium, 2008, 43(6): 615-621.
- [3] Malgaroli A, Milani D, Meldolesi J, et al. Fura-2 measurement of cytosolic free Ca²⁺ in monolayers and suspensions of various types of animal cells[J]. The Journal of cell biology, 1987, 105(5): 2145-2155.
- [4] Nakamura I, Nakai Y, Izumi H. Use of fura-2/AM to measure intracellular free calcium in *Selenomonas ruminantium*[J]. The Tohoku Journal of Experimental Medicine, 1996, 179(4): 291-294.
- [5] Barreto-Chang O L, Dolmetsch R E. Calcium imaging of cortical neurons using Fura-2 AM[J]. Journal of visualized experiments: JoVE, 2009 (23): 1067.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA