

Product Data Sheet

Product Name: Mag-Fura-2 AM

Cat. No.: GB30290

Chemical Properties

Cas. No. 130100-20-8

Formula $C_{30}H_{30}N_2O_{19}$ M.Wt 722.56

Solubility Storage Store at -20°C, minimize light exposure

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.

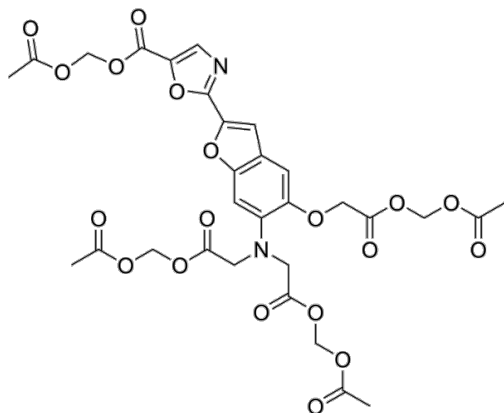
Caution: Product has not been fully validated for medical applications. For research use only.

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Structure



Protocol

Evaluation of calcium content using Mag-Fura-2 AM ^[1]

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

1. Prepare dyeing solution

(1) Prepare stock solution: Prepare Mag-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. Please adjust the concentration of the working fluid according to the actual situation, and

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prepare it now to avoid repeated freezing and thawing. Notice: The working solution must be prepared fresh on the day of the experiment. Before use, Mag-Fura-2 AM solution needs to be rewarmed and centrifuged.

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) Incubate the cells at the required temperature (25°C-37°C) for 10 minutes to allow the ionic gradient to reach stability and equilibrium. Take 4µl of the Mag-Fura-2 AM stock solution and 5µl of 20% Pluronic® F-127, add them to 1ml of cell culture medium, and vortex-mix vigorously to obtain a homogeneous probe dispersion solution. Take 250µl of the prepared probe dispersion solution and add it to 750µl of cell-containing culture medium, mix well. At this point, a working solution with a final concentration of 1-5µM is obtained based on the initial mother solution concentration. Incubate the probe for 15-60 minutes according to the requirements. Wash the cells 3 times, and then continue to incubate for 30 minutes to ensure that the probes in the cells are completely de-esterified.

Note:

1) The recommended working concentration of Mag-Fura-2 AM in most cells is 1-5µM, 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 sulfapyrazone (0.1-0.25mM) to the cell culture medium. to reduce the leakage level of the de-esterification probe. The stock solution of probenecid or sulfapyrazone 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 Mag-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;

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

(4) Select the appropriate instrument and conduct fluorescence measurement ($E_x/E_m=336/503\text{nm}$).

3. Cell adhesion staining

(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) Incubate the cells at the required temperature (25°C - 37°C) for 10 minutes to allow the ionic gradient to reach stability and equilibrium. Take $4\mu\text{l}$ of the Mag-Fura-2 AM stock solution and $5\mu\text{l}$ of 20% Pluronic® F-127, add them to 1ml of cell culture medium, and vortex-mix vigorously to obtain a homogeneous probe dispersion solution. Take $250\mu\text{l}$ of the prepared probe dispersion solution and add it to $750\mu\text{l}$ of cell-containing culture medium, mix well. At this point, a working solution with a final concentration of $1\text{-}5\mu\text{M}$ is obtained based on the initial mother solution concentration. Incubate the probe for 15-60 minutes according to the requirements. Wash the cells 3 times, and then continue to incubate for 30 minutes to ensure that the probes in the cells are completely de-esterified.

(4) Select the appropriate instrument and conduct fluorescence measurement ($E_x/E_m=336/503\text{nm}$).

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) Mag-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] Gurney A M, Drummond R M, Fay F S. Calcium signalling in sarcoplasmic reticulum, cytoplasm and mitochondria during activation of rabbit aorta myocytes[J]. Cell Calcium, 2000, 27(6): 339-351.

Background

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Mag-Fura-2 AM is a fluorescent dye which is useful for measuring changes in cytosolic Mg^{2+} with $E_x/E_m=336/503nm$ [1]. Mag-Fura-2 AM can be used to study the characteristics of Mg^{2+} transport in the brush border membrane vesicles of the ileum of animals[2]. Mag-Fura-2 AM is used as a Ca^{2+} -sensitive fluorescent indicator to detect the calcium signal transduction in the sarcoplasmic reticulum, cytoplasm and mitochondria during the activation process of rabbit aortic smooth muscle cells[3].

References:

- [1] Grubbs R D, Walter A. Determination of cytosolic Mg^{2+} activity and buffering in BC3H-1 cells with mag-fura-2[J]. Molecular and cellular biochemistry, 1994, 136(1): 11-22.
- [2] Jüttner R, Ebel H. Characterization of Mg^{2+} transport in brush border membrane vesicles of rabbit ileum studied with mag-fura-2[J]. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1998, 1370(1): 51-63.
- [3] Gurney A M, Drummond R M, Fay F S. Calcium signalling in sarcoplasmic reticulum, cytoplasm and mitochondria during activation of rabbit aorta myocytes[J]. Cell Calcium, 2000, 27(6): 339-351.

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