

PRODUCT INFORMATION



Fura-2 Leakage Resistant AM Item No. 34993

Formal Name: bis(acetoxymethyl) 2,2'-((2-(5-((acetoxymethoxy)carbonyl)oxazol-2-yl)-5-(2-(2-(bis(2-(acetoxymethoxy)-2-oxoethyl)amino)-5-(3-oxo-3-((prop-1-en-2-yloxy)methoxy)propyl)phenoxy)ethoxy)benzofuran-6-yl)azanediyloxy)diacetate

Synonyms: Fura-2 Leakage Resistant Acetoxymethyl ester, Fura-2 LR Acetoxymethyl ester, Fura-2 LR AM, Fura-PE3

MF: C₄₉H₅₃N₃O₂₈

FW: 1,131.9

Purity: ≥95%

Ex./Em. Max: 340 and 380/505 nm

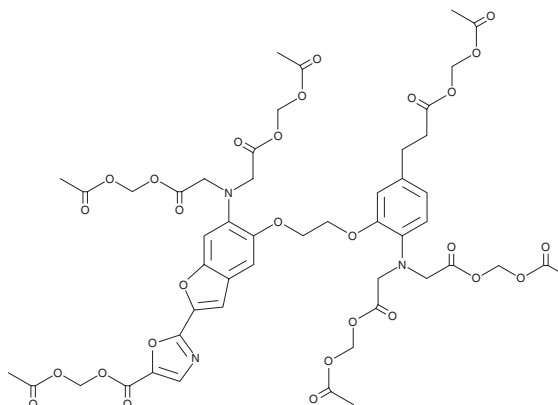
Supplied as: A solid

Storage: -20°C

Stability: ≥4 years

Special Conditions: Protect from light and moisture

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.



Description

Fura-2 leakage resistant AM is a cell-permeable, ratiometric fluorescent calcium indicator. It binds to calcium ($K_d = 145$ nm) and displays excitation maxima of 340 and 380 nm when calcium-bound or calcium-free, respectively, when the emission is fixed at 505 nm. Fura-2 leakage resistant is also available in a cell-impermeable form (Item No. 34994).

Assay Protocol

Note: Allow all reagents to warm to room temperature before proceeding.

1. Add 10 ml of assay buffer to a 15 or 50 ml conical tube.

Note 1: HEPES-buffered Hank's balanced salt solution (HBSS), pH 7.2-7.4, is recommended, although other buffers can be used.

2. Add 100 μ l of a 100X Pluronic™ F-127 solution (1-50% w/v) to the conical tube*. Pluronic™ F-127 is a biocompatible surfactant used to ensure equitable dye distribution and cellular loading.
 - a. Optional: Add 100 μ l of 2 mM probenecid stock solution to the conical tube. Probenecid (Item No. 14981) is an anion transport inhibitor used to improve intracellular dye retention. Use of probenecid is recommended, but not required, for all cell types and dyes.

*Final working concentration of Pluronic™ F-127 should be between 0.01 and 0.5% w/v. User should optimize the concentration of Pluronic™ F-127 to suit experimental requirements.

Note 2: Probenecid is an inhibitor or agonist of multiple ion channels and may have undesirable cellular effects that could affect dye performance.

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

WARRANTY AND LIMITATION OF REMEDY
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3. Vortex conical tube briefly to mix.
4. Dissolve 50 μg of Fura-2 Leakage Resistant AM in 25 μl of DMSO and vortex dye tube briefly to mix.
5. Centrifuge dye tube briefly to collect all contents at the tube bottom.
6. Add entire contents of dye tube to the conical tube containing the assay buffer solution to make the dye loading solution.
7. Vortex conical tube briefly to mix.

Note 3: The dye loading solution should be used within two hours for best results.

8. Remove cell culture medium and add dye loading solution. Recommended volumes are:
 - a. 35 mm dish or 6-well plate: 1.5 ml/dish or well
 - b. 96-well plate: 100 μl /well
 - c. 384-well plate: 20 μl /well

Note 4: To prevent cell detachment or if using suspension cells, the dye loading solution can be added directly to the media-containing wells. User must double the component concentrations to achieve the same final concentration of all reagents.

9. Incubate cells with the dye loading solution at 37°C for 60 minutes.
10. Read fluorescence using a plate reader at excitation and emission wavelengths of 340 or 380 and 505 nm, respectively.

Or

Image using a fluorescence microscope with filters compatible for Fura-2.
 - a. Optional: To minimize extracellular background, the dye loading solution can be replaced with assay buffer containing 1X probenecid solution.

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