Fluo-4 AM

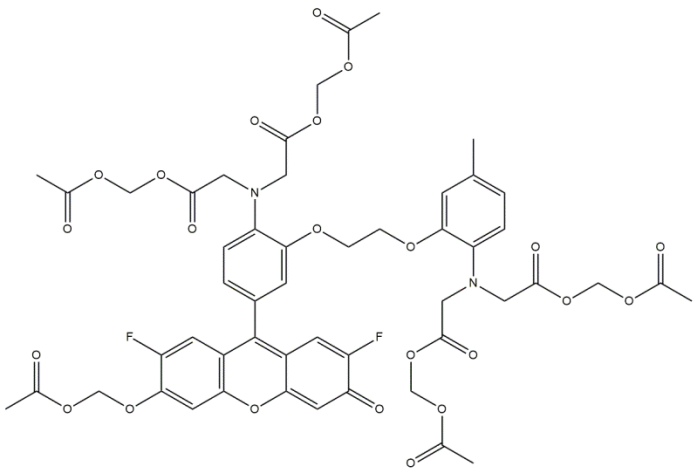
05/21

ALTERNATE NAMES: Calcium ($Ca2^+$) indicator; Fluo-4 membrane permeable; Fluo-4 acetoxymethyl ester; Fluorescent Calcium indicator

CATALOG #:

B3146-5PK	5 x 50 µg
B3146-500	500 µg

STRUCTURE:



MOLECULAR FORMULA: $C_{51}H_{50}F_2N_2O_{23}$

MOLECULAR WEIGHT: 1097

CAS NUMBER: 273221-67-3

APPEARANCE: Red orange powder

PURITY: ≥ 95%

SOLUBILITY: ~1 mg/ml in DMSO

DESCRIPTION: Fluo-4 is a widely used green fluorescent, intracellular calcium (Ca^{2+}) indicator. Fluo-4 AM is the membrane permeable form of the calcium indicator. Masking the negative charge using non-polar, ester-linked moieties (AM ester) allows the molecule to enter cells through passive diffusion. Once inside the cell, ubiquitous intracellular esterase enzymes promote rapid hydrolysis of the AM ester leaving the active, highly polar form of the indicator trapped inside the cell. It binds to calcium with a K_d value of 355 nM and displays excitation/emission maxima of 490/515 nm, respectively. It is used to measure intracellular Ca^{2+} flux in high throughput screening and fluorescence microscopy applications.

STORAGE TEMPERATURE: -20 ºC. Store in the dark. Product is light sensitive. Protect from air. Store under desiccating conditions.

HANDLING: Do not take internally. Wear gloves and mask when handling the product! Avoid contact by all modes of exposure.

PROTOCOL:

User Supplied Reagents:

- DMSO
- Pluronic F-127 solution
- Probenecid solution (optional)
- TRS solution (optional)
- HEPES-buffered Hank's Balanced Salt Solution

Procedure:

The following protocol provides general guidelines. All the loading conditions (concentrations, temperature and time) should be determined for specific assays, applications, and instrumentation.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 10 ml of assay buffer to a 15 ml conical tube. HEPES-buffered Hank's Balanced Salt Solution (pH 7.2-7.4) is the most commonly used assay buffer, although other buffers can also be used.
3. Add Pluronic F-127 solution to the assay buffer in conical tube. Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
4. Optional: Add Probenecid solution to the assay buffer. Probenecid is an anion transport inhibitor that improves intracellular dye retention. Although it is not required for all cell types and dyes, it is recommended in most cases to optimize assay performance.
5. Optional: Add TRS solution. TRS is a membrane impermeant dye useful for masking extracellular fluorescence.
   Note: Caution is advised when using probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest.
6. Vortex the conical tube briefly to mix.
7. Dissolve Fluo-4 AM in 25 µl of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator dye tube to assay buffer solution to make a dye loading solution. The dye loading solution should be used within 2 h of dye addition for best results.
8. Vortex dye loading solution briefly to mix.
9. Remove the cell culture medium and add dye loading solution. Recommend volumes are: 35 mm dish or 6-well plate, 1.5 ml; 96-well plate, 100 µl; 384-well plate, 20 µl. When cells are sensitive to solution exchanges and will either be aspirated (suspension cells) or detach (loosely adherent cells) during wash steps, we recommend performing assays without removing any solution (no wash format) by adjusting the concentrations of reagents in the dye loading solution so that when everything is added to the wells, the final concentrations of all reagents are the same. If a no wash format is indicated for your application, we recommend doubling the concentration of all reagents in your dye loading buffer.
10. Incubate in a cell culture incubator at 37 ºC for 60 min.
11. Read the fluorescence using a plate reader (Excitation/Emission: 490 nm/515 nm) or image using a fluorescence microscope (using filters for GFP or fluorescein). To minimize extracellular background, dye loading solution can be replaced with assay buffer containing 1X probenecid solution (optional) and/or 1X TRS solution (optional).

REFERENCES:

1. Gee, K.R., Brown, K.A., Chen, W.N., et al. Chemical and physiological characterization of fluo-4 Ca(2+)-indicator dyes. Cell Calcium. 27(2):97-106 (2000).
2. Bovo, E., Dvornikov, A.V., Mazurek, S.R., et al. Mechanisms of Ca^{2+} handling in zebrafish ventricular myocytes. Pflugers Arch. 2013 Dec;465(12):1775-84 (2013).
3. Rodriguez, A.L., Grier, M.D., Jones, C.K., et al. Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 reveals chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity. Mol Pharmacol. 78(6):1105-23 (2010).
4. Xiang Z., Thompson, A.D., Brogan, J.T., et. al. The Discovery and Characterization of ML218: A Novel, Centrally Active T-Type Calcium Channel Inhibitor with Robust Effects in STN Neurons and in a Rodent Model of Parkinson's Disease. ACS Chem Neurosci. 21;2(12):730-742 (2011).

RELATED PRODUCTS:

IPG-1 TMA+ Salt (Cat. No. B3141)
ING-2 TMA+ Salt (Cat. No. B3138)
IPG-2 AM (Cat. No. B3142)
FURA-5F/AM (Cat. No. 9551)
IPG-1 AM (Cat. No. B3140)

DISCLAIMER: ***FOR RESEARCH USE ONLY! Not to be used on humans.***