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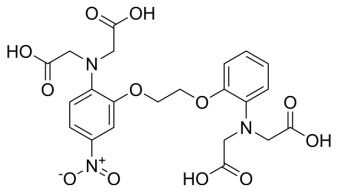
5-Nitro BAPTA

Cat. No.:	HY-D1636
CAS No.:	124251-83-8
Molecular Formula:	$C_{22}H_{23}N_3O_{12}$
Molecular Weight:	521.43
Target:	Fluorescent Dye
Pathway:	Others
Storage:	Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description

5-Nitro BAPTA is a calcium chelator, combinded with 2-Me-substituted TM (as a fluorescent moiety), can be used to form a red fluorescent probe (CaTM-2 AM), for imaging of cytoplasmic Ca^{2+} in cultured living cells. 5-Nitro BAPTA is a building block used in the synthesis of Ca^{2+} specific chelators, Ca^{2+} buffers, and fluorescent Ca^{2+} indicators[1][2].

In Vitro

5-Nitro BAPTA, designed to a red fluorescent probe for cytoplasmic Ca^{2+} with strong emission in the long-wavelength region [1].

General procedure for fluorescence imaging of cultured Hela cells[1]:

1.Plate cells onto a 35-mm poly-L-lysinecoated glass-bottomed dish (Matsunami) in DMEM supplemented with 10% (v/v) fetal bovine serum, 1% penicillin and 1% streptomycin.

2. Remove DMEM, wash the dish with HBSS 3 times, and then add CaTM-2 AM (3 μM) in Hanks' Balanced Salt Solution (HBSS) containing 0.3% DMSO as a cosolvent.

3. Incubate at 37°C for 30 min, remove medium and wash dishes with HBSS 3 times. The cells can be observed in HBSS.

4. Capture fluorescence images with excitation and emission wavelength of 590/610–680 nm.

General procedure for fluorescence imaging of slices[1]:

1. Incubate slide cultures with 2 mL dye solution at 37 °C for 40 min. The dye solution is artificial cerebrospinal fluid (aCSF) containing 10 μM CaTM-2 AM, 0.01% Pluronic F-127, and 0.005% Cremophor EL. aCSF consisted of : 126 mM NaCl, 26 mM $NaHCO_3$, 3.5 mM KCl, 1.24 mM NaH_2PO_4, 1.3 mM $MgSO_4$, 1.2 mM $CaCl_2$, and 10 glucose.

2. Wash slieds with aCSF three times and recover in 2 mL aCSF at 37 °C for 45 min, during which 2 μL of 1 mM Acridine orange was added to the aCSF at time 40 min.

3. Transferre slice cultures into a recording chamber heated at 35 °C and continuously perfused with aCSF at 2 mL/min.

4. Acqure images at 10 frames/s with a Nipkowdisk confocal unit (CSUX-1, Yokogawa Electric, Tokyo, Japan), cooled CCD camera (iXon DU897, Andor, Belfast, UK), a water-immersion objective lens (16×, 0. NA, Nikon, Tokyo, Japan), and image acquisition software (Solis, Andor Technology, Belfast, UK).

5. Set the excitation wavelength to 488 nm (7 mW) and 568 nm (15 mW) for Acridine orange and CaTM-2 with an argon-krypton laser (641-YB-A01; Melles Griot, Carlsbad, CA, USA) and set the emission wavelength to 520-535 nm and 617-673 nm band-pass emission filters, respectively.

6. Analysis data with custom-made software written in Microsoft Visual Basic.

7. Calculate fluorescence change ΔF/F as (Ft-F0)/F0, where Ft is the fluorescence intensity at frame time t, and F0 is the average baseline.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Takahiro Egawa, et al. Red Fluorescent Probe for Monitoring the Dynamics of Cytoplasmic Calcium Ions†. Angew Chem Int Ed. 2013, 52(14):3874-3877.

[2]. Jones, et al. Purification and labeling of extracellular vesicles using a mixed mode resin composition: World Intellectual Property Organization, WO2019133842[P]. 2019-07-04.

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