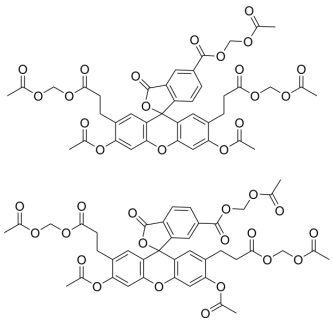
BCECF-AM

Cat. No.:	HY-101883
CAS No.:	117464-70-7
Molecular Formula:	$C_{40}H_{36}O_{19}$
Molecular Weight:	820.7
Target:	Fluorescent Dye
Pathway:	Others
Storage:	-20°C, protect from light

* The compound is unstable in solutions, freshly prepared is recommended.



BIOLOGICAL ACTIVITY

Description	BCECF-AM is a cell membrane permeable compound widely used as a fluorescent indicator for intracellular pH.
In Vitro	Fully treated cells show hydrogenosomes with an electron-dense deposit which aggregates to a variable extent.The staining is seen in the interior of hydrogenosomes in some instances. It is also observed by microscopy that the K^+/H^+ ionophor nigericin does not inhibit hydrogenosomal loading with BCECF-AM[1]. The pH-sensitive fluorescent dyes to measure cytosolic pH. 1.Prepare a 2 to 20 mM stock solution of BCECF-AM in DMSO. 2.Prepare a 5-50 μM BCECF-AM dye-loading solution in buffer solutions (HHBS or PBS). 3. Add 1000 μL/well (6-well plate),100 μL/well (96-well plate) or 25 μL/well (384-well plate) BCECF-AM dye-loading solution into the cell plate. 4. Incubate the dye-loading plate in a cell incubator for 30-60 minutes. 5. Wash and replace the dye-loading solution with buffers. 6. Run the pH assay by monitoring the fluorescence at E_x/E_m = 490/535 nm or 430/535 nm for ratio measurements. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [2]	PASMCs are placed in a laminar flow cell chamber perfused with HBSS with pH adjusted to 7.4. pH_i is measured in cells incubated with the membrane permeant (acetoxymethyl ester) form of the pH-sensitive fluorescent dye BCECF-AM for 60 min at 37°C under an atmosphere of 20% O_2-5% CO_2. Cells are then washed with HBSS for 15 min at 37°C to remove extracellular dye and allow complete de-esterification of cytosolic dye. Ratiometric measurement of BCECF fluorescence is performed on a workstation consisting of a Nikon TSE 100 Ellipse inverted microscope with epi-fluorescence attachments. The light beam from a xenon arc lamp is filtered by interference filters at 490 and 440 nm, and focused onto the PASMCS under examination via a 20× fluorescence objective. Light emitted from the cell at 530 nm is returned through the objective and detected by an imaging camera. An electronic shutter is used to minimize photobleaching of dye. Protocols are executed and data collected on-line with InCyte software. pH_i is estimated from in situcalibration after each experiment. Cells are perfused with a solution containing (in mM): 105 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 10 glucose, 20 HEPES-Tris and 0.01 nigericin to allow pH_i to equilibrate to external pH. A two point calibration is created from fluorescence measured as pH_i is adjusted with KOH from 6.5 to 7.5. Intracellular H^+ ion concentration ($[H^+]i$) is determined from pH_i using the formula: $pH_i = -\log([H^+]i)$. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Plant Physiol. 2023 Jun 13;kiad339.
- Am J Physiol Cell Physiol. 2020 Apr 1;318(4):C806-C816.
- J Aquat Plant Manage. 2023 Jul 19, 61: 55–62.

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REFERENCES

[1]. Scott DA, et al. Analysis of the uptake of the fluorescent marker 2',7'-bis-(2-carboxyethyl)-5(and-6)-carboxyfluorescein (BCECF) by hydrogenosomes in Trichomonas vaginalis. Eur J Cell Biol. 1998 Jun;76(2):139-45.

[2]. Clark Undem, et al. Endothelin-1 Augments Na+/H+ Exchange Activity in Murine Pulmonary Arterial Smooth Muscle Cells via Rho Kinase. PLoS One. 2012; 7(9): e46303.

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