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FRONTIERS IN LIFE SCIENCE

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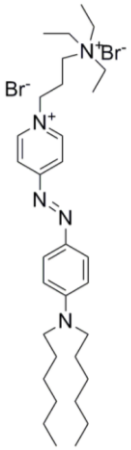
Ap3, SHG Imaging Dye

Product Background

Ap3 is a newly synthesized novel molecule for second harmonic generation (SHG) imaging. SHG is a nonlinear optical process, the interaction of two photons with a nonlinear material generates photons with twice the energy. SHG imaging is a powerful tool to visualize cell and tissue structure and function. However, most of dyes for SHG imaging emit strong fluorescence signals in addition to SHG signals. The fluorescence emission from dyes disturbs multimodal imagining as well as SHG signal imaging. Ap3 is the first novel dye designed specifically for SHG imaging with virtually no fluorescence signals, improved photostability and less phototoxicity. Ap3 enables us to detect true SHG signals and realizes multimodal imaging.

Description

Catalog Number: FDV-0008
Lot Number: see vial label
Size: 1 mg
Chemical structure:



Chemical name: (E)-4-((4-(dihexylamino)phenyl)diazenyl)-1-(3-(triethylammonio)propyl)pyridin-1-ium bromide
CAS No.: 1638495-90-5
Molecular formula: $C_{32}H_{55}Br_2N_5$
Molecular weight: 669.6206
Solubility: Soluble in water
Purity: ≧98%
License: This product has been commercialized with a license from Keio University and University of Tsukuba.
Warning: Research use only. Not for use in humans.

Reconstitution and Storage

Reconstitution: Reconstitute at 10 mM as stock solution in an appropriate buffer (e.g. 125 mM NaCl, 5 mM KCl, 10 mM dextrose, 10 mM HEPES, 1 mM $MgCl_2$, 2 mM $CaCl_2$, pH7.3).
Storage: Store at -20℃.

Instruction

For extracellular loading of cultured cells

1. Plate your cells with appropriate cell density in glass-bottom dishes.

2. Dilute a Ap3 stock solution in an appropriate buffer (e.g. 125 mM NaCl, 5 mM KCl, 10 mM dextrose, 10 mM HEPES, 1 mM $MgCl_2$, 2 mM $CaCl_2$, pH7.3).

3. Remove culture medium from glass-bottom dishes and apply diluted Ap3 (e.g. 200 µl ~ 1 ml) to cells at a final concentration of 20 µM.

4. Incubate cells for a few mins.

5. Generate the SHG signals with 950 nm laser illumination and observe it with 465 – 485 nm band pass filter through two-photon microscopy system.

For intracellular loading of neurons in acute brain slices

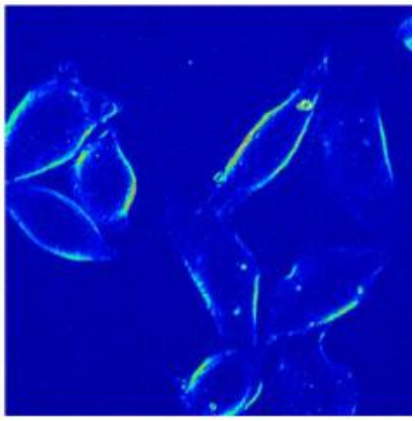
1. Prepare 300 µm-thick acute cortical brain slices.

2. Dilute a Ap3 stock solution in an internal solution (e.g. 10mM NaCl, 10mM KCl, 135mM $KMeSO_4$, 2.5mM MgATP, 0.3mM NaGTP and 10mM HEPES, pH 7.3).

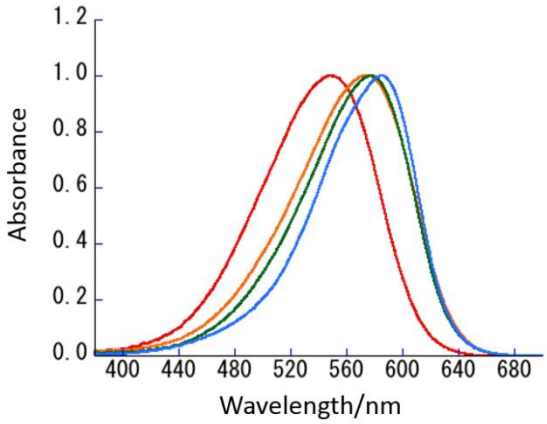
3. Load the target neuron with Ap3 through patch-clamp pipette (loading time may vary depending on the size of the pipette etc.).

4. Generate the SHG signals with 950 nm laser illumination and observe it with 465 – 485 nm band pass filter through two-photon microscopy system.

※For SHG imaging, the detection system is required to set on the opposition side of the objective lens. Besides, the installation of photomultiplier tube (PMT) is preferable on the detection side.



SHG signals obtained from cultured CHO cells loaded with 20 µM Ap3.



Single photon absorption spectra of Ap3
Red line; THF
Orange line; DMSO
Green line; Methanol
Blue line; Distilled water

Reference

1) Nuriya M, et al, Nat Commun, 7:11557, 2016

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2018/08/02