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FRONTIERS IN LIFE SCIENCE

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Ap3, SHG Imaging Dye

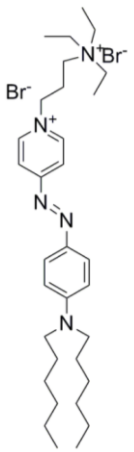
Catalog NO. FDV-0008

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 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
Size: 1 mg
Formulation: $C_{32}H_{55}Br_2N_5$
Molecular weight: 669.62 g/mol
Chemical Structure: see right figure
Solubility: Soluble in water
SHG information:
SHG inducing laser: 950 nm laser
Emission filter: 465-485 nm



Reconstitution and Storage

Reconstitution: Stock solution recommended concentration 10 mM 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 (powder): Store powder at -20°C
Storage (solution): After reconstitution, aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.

Important Notice

Ap3 is a specific dye for SHG imaging without emission of fluorescent signal. For detection of Ap3's SHG signal, two-photon microscopy equipped SHG detector system is required. Furthermore, **the SHG 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.

How to use

General procedure for extracellular loading of cultured cells

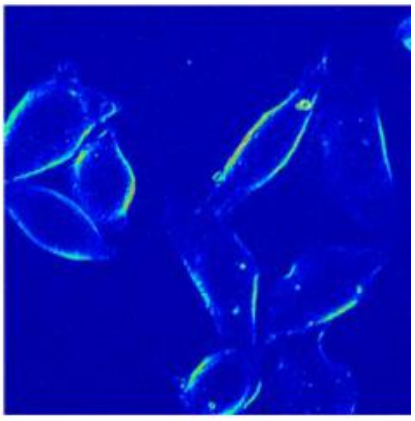
1. Plate your cells with appropriate cell density in glass-bottom dishes.
2. Dilute a Ap3 stock solution in an appropriate buffer (e.g. 125 mM NaCl, 5 mM KCl, 10 mM dextrose, 10 mM HEPES, 1 mM $MgCl_2$, 2 mM $CaCl_2$, pH7.3).
3. Remove culture medium from glass-bottom dishes and apply diluted Ap3 (e.g. 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.

General procedure for intracellular loading of neurons in acute brain slices

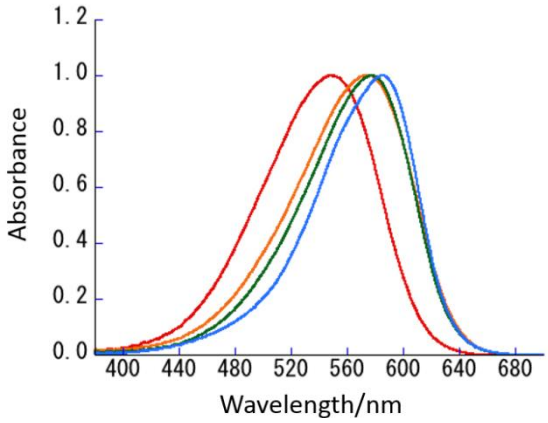
1. Prepare 300 µm-thick acute cortical brain slices.
2. Dilute a Ap3 stock solution in an internal solution (e.g. 10 mM NaCl, 10 mM KCl, 135 mM $KMeSO_4$, 2.5 mM MgATP, 0.3 mM NaGTP and 10m M HEPES, pH 7.3).
3. Load the target neuron with Ap3 through patch-clamp pipette (loading time may vary depending 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.

NOTE: Empirically optimize and determine the concentration and incubation time of Ap3 for your experiments.

Reference Data



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 *et al., Nat. Commun.*, **7**, 11557 (2016) Multimodal two-photon imaging using a second harmonic generation-specific dye
2. Mizuguchi *et al., iScience*, **9**, 359-366 (2018) High-Resolution Plasma Membrane-Selective Imaging by Second Harmonic Generation

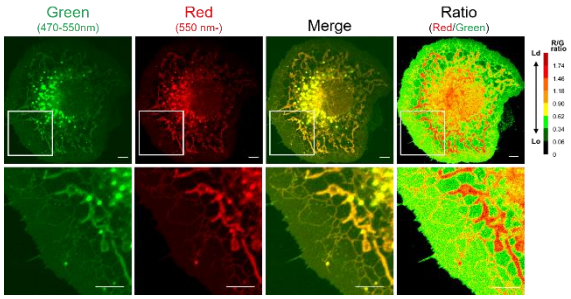
Related products

LipiORDER™ <Membrane Lipid Order Imaging Dye>

LipiORDER™ is a solvatochromic dye for membrane lipid order imaging. LipiORDER™ exhibits green fluorescence with Lo phase and exhibits red fluorescence with Ld phase. The ratiometric analysis (F_{red}/F_{green}) enables the quantitative visualization of membrane lipid order.

Catalog No. FDV-0041
Size 0.1 mg
Features

- Recommended Ex/Em: ~405 nm / 500-550 nm (Green channel) and 550-650 nm (Red channel)
- Enable to quantitatively monitor lipid order on plasma and inner membranes in live cells
- Highly photostable and cellularly stable compared with similar conventional dyes.

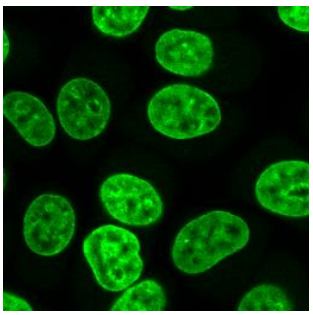


NucleoSeeing™ <Live Nucleus Green>

NucleoSeeing™ is DNA-responsive green dye for monitoring cell nucleus in live cells. As it shows low cytotoxicity and phototoxicity, it is very suitable for long-term live imaging of cell nucleus.

Catalog No. FDV-0029
Size 0.1 mg
Features

- Easy and quick procedure
- Compatible with 10% FBS
- Validated for both adherent cells and floating cells
- Little influence on cellular functions
- Ex/Em: 488 nm/520 nm (commercial FITC filters are available)

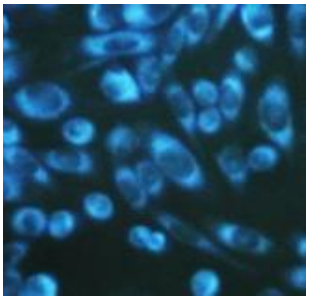


CytoSeeing™ <Reversible Cytoplasm Blue>

CytoSeeing™ is a reversible blue cytoplasm-staining dye for monitoring cell morphology. It allows observing cell structure and to reuse the cells after removing dye.

Catalog No. FDV-0017
Size 1 mg
Features

- Easy and quick staining less than 10 min
- Washable, reversible staining
- Validated for both adherent cells and suspension cells
- Little influence on cellular functions
- Ex/Em: 345 nm/456 nm

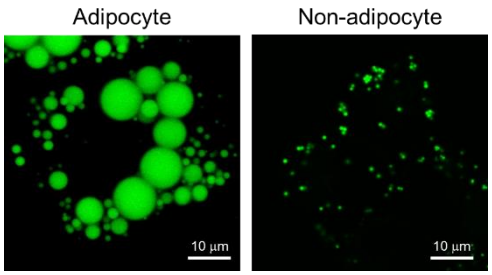


LipiDye™ II <Live Imaging>

LipiDye™ II is a highly sensitive lipid droplet staining dye with extremely photostable property. This dye is the second generation of our previous reagent, LipiDye™. This dye allows us to detect small lipid droplets (<1 μm) in non-adipocytes and to apply into long-term live cell imaging for dynamic lipid droplet movements.

Catalog No. FDV-0027
Size 0.1 mg
Features

- Recommended Ex/Em:400-500 nm / 490-550 nm
- Enable to detect <1 μm lipid droplets
- Suitable for long-term live cell imaging
- Extremely photostable compared with conventional dyes
- Compatible with both live and fixed cells

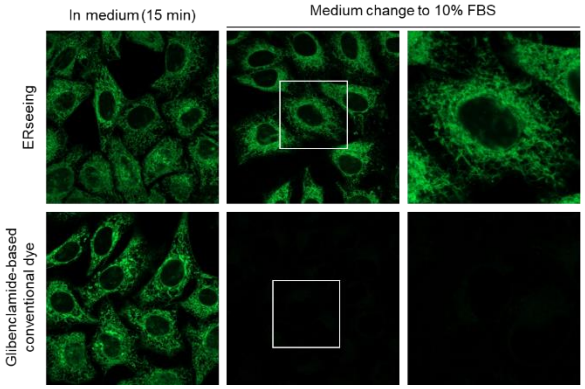


ERseeing™ <Endoplasmic reticulum Green>

ERseeing™ is a novel type of ER-staining dye and shows little pharmacological effects compared with conventional glibenclamide-based ER dyes. ERseeing is irreversible staining and is compatible with medium change for long-term imaging.

Catalog No. FDV-0038
Size 10 nmol
Features

- Recommended Ex/Em: 509 nm/524 nm
- Less pharmacological effect on ER proteins
- Suitable for long-term live cell imaging



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