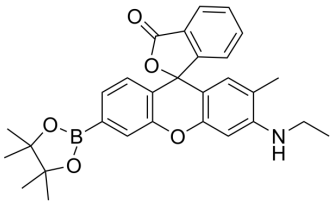
NucPE1

Cat. No.:	HY-101859
CAS No.:	1404091-23-1
Molecular Formula:	$C_{29}H_{30}BNO_5$
Molecular Weight:	483.36
Target:	Reactive Oxygen Species
Pathway:	Immunology/Inflammation; Metabolic Enzyme/Protease; NF-κB
Storage:	4°C, protect from light, stored under nitrogen * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light, stored under nitrogen)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 25 mg/mL (51.72 mM; Need ultrasonic)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	2.0689 mL	10.3443 mL	20.6885 mL
	5 mM	0.4138 mL	2.0689 mL	4.1377 mL
	10 mM	0.2069 mL	1.0344 mL	2.0689 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.17 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

NucPE1 (Nuclear Peroxy Emerald 1) is a nuclear-localized fluorescent hydrogen peroxide that is specifically localized to cellular nuclei without appended targeting moieties.

In Vitro

NucPE1 features two major visible region absorptions (λ_{abs}=468 nm, ε=27,300 $M^{-1}cm^{-1}$; λ_{abs}=490 nm, ε=26,000 $M^{-1}cm^{-1}$) and a weak emission (λ_{em}=530 nm, Φ=0.117). Reaction of NucPE1 with H_2O_2 triggers a fluorescence increase upon its conversion to fluorophore NucPE1, which possesses one major absorption band at 505 nm (ε=19,100 $M^{-1}cm^{-1}$) with enhanced emission (λ_{em}=530 nm, Φ=0.626). NucPE1 selectively accumulates in the nuclei of a variety of mammalian cell lines as well as in whole model organisms like C. clegans, where it can respond to subcellular changes in H_2O_2 fluxes[1].

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

In Vivo

NucPE1 maintains the ability to selectively target nuclei in vivo. NucPE1 imaging reveals a reduction in nuclear H_2O_2 levels in worms overexpressing sir-2.1 compared to wildtype congeners, supporting a link between this longevity-promoting sirtuin protein and enhanced regulation of nuclear ROS pools[1].

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PROTOCOL

Cell Assay [1][2]

Excitation of NucPE1-loaded cells at 514 nm is carried out with an Ar laser and emission is collected using a META detector between 522–554 nm. Excitation of Hoechst 33342 is carried out using a MaiTai two-photon laser at 780-nm pulses and emission is collected between 436–501 nm. All images in an experiment are collected simultaneously using identical microscope settings. Image analysis is performed in Image J. The background fluorescence is measured in a data set by selecting an ROI outside of the cells. This background number is then used to set the threshold of the image and thereby select for NucPE1 signal. The mean fluorescence intensity of this signal is then measured and utilized as the fluorescent signal for that particular image. The same threshold settings are utilized for all images in an experiment[1]. The measurement of nuclear H_2O_2 is achieved using NucPE1. The cells are incubated for 45 min at 10 μM NucPE1 in the dark. The incubation is followed by ishing and analysis by flow cytometry as explained above. Mitochondrial ROS are measured using MitoSOX probe. The cells are incubated with 5 μM MitoSOX for 15 min in the dark. The incubation is followed by ishing and analysis by flow cytometry[2]. Live imaging of the NucPE1 probe is carried out using excitation at 488 nm with an argon laser, and emission is collected using a META detector at about 520 nm. The Hoechst dye is incubated together with NucPE1. When multiple staining of NucPE1 and PO1 is performed, the multitracking mode of scanning is applied for acquisition of the images[2].

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CUSTOMER VALIDATION

- FASEB J. 2020 Sep;34(9):11474-11487.

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REFERENCES

[1]. Stanicka J, et al. NADPH oxidase-generated hydrogen peroxide induces DNA damage in mutant FLT3-expressing leukemia cells. J Biol Chem. 2015 Apr 10;290(15):9348-61.

[2]. Dickinson BC, et al. A nuclear-localized fluorescent hydrogen peroxide probe for monitoring sirtuin-mediated oxidative stress responses in vivo. Chem Biol. 2011 Aug 26;18(8):943-8.

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