

# **Data Sheet**

Product Name:	NucPE1	
Cat. No.:	CS-7002	
CAS No.:	1404091-23-1	
Molecular Formula:	C <sub>29</sub> H <sub>30</sub> BNO <sub>5</sub>	
Molecular Weight:	483.36	
Target:	Reactive Oxygen Species	
Pathway:	Immunology/Inflammation; Metabolic Enzyme/Protease; NF-кB	+0
Solubility:	DMSO : 25 mg/mL (51.72 mM; Need ultrasonic)	1

## **BIOLOGICAL ACTIVITY:**

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 ( $\lambda_{abs}$ =468 nm,  $\epsilon$ =27,300 M <sup>-1</sup>cm<sup>-1</sup>;  $\lambda_{abs}$ =490 nm,  $\epsilon$ =26,000 M<sup>-1</sup>cm<sup>-1</sup>) and a weak emission ( $\lambda_{em}$ =530 nm,  $\Phi$ =0.117). Reaction of NucPE1 with H<sub>2</sub>O<sub>2</sub> triggers a fluorescence increase upon its conversion to fluorophore NucPE1, which possesses one major absorption band at 505 nm ( $\epsilon$ =19,100 M<sup>-1</sup>cm<sup>-1</sup>) with enhanced emission ( $\lambda_{em}$ =530 nm,  $\Phi$ =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<sub>2</sub>O<sub>2</sub> fluxes<sup>[1]</sup>. *In Vivo:* NucPE1 maintains the ability to selectively target nuclei *in vivo*. NucPE1 imaging reveals a reduction in nuclear H<sub>2</sub>O<sub>2</sub> 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<sup>[1]</sup>.

### PROTOCOL (Extracted from published papers and Only for reference)

**Cell Assay:** <sup>[1][2]</sup>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<sup>[1]</sup>. The measurement of nuclear H<sub>2</sub>O<sub>2</sub> is achieved using NucPE1. The cells are incubated for 45 min at 10  $\mu$ M 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  $\mu$ M MitoSOX for 15 min in the dark. The incubation is followed by ishing and analysis by flow cytometry<sup>[2]</sup>. 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<sup>[2]</sup>.

#### **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.

## **CAIndexNames:**

Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3'-(ethylamino)-2'-methyl-6'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-

## SMILES:

O=C1OC2(C3=C(OC4=C2C=CC(B5OC(C)(C)C(C)(C)O5)=C4)C=C(NCC)C(C)=C3)C6=C1C=CC=C6

#### Caution: Product has not been fully validated for medical applications. For research use only.

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