funakoshi

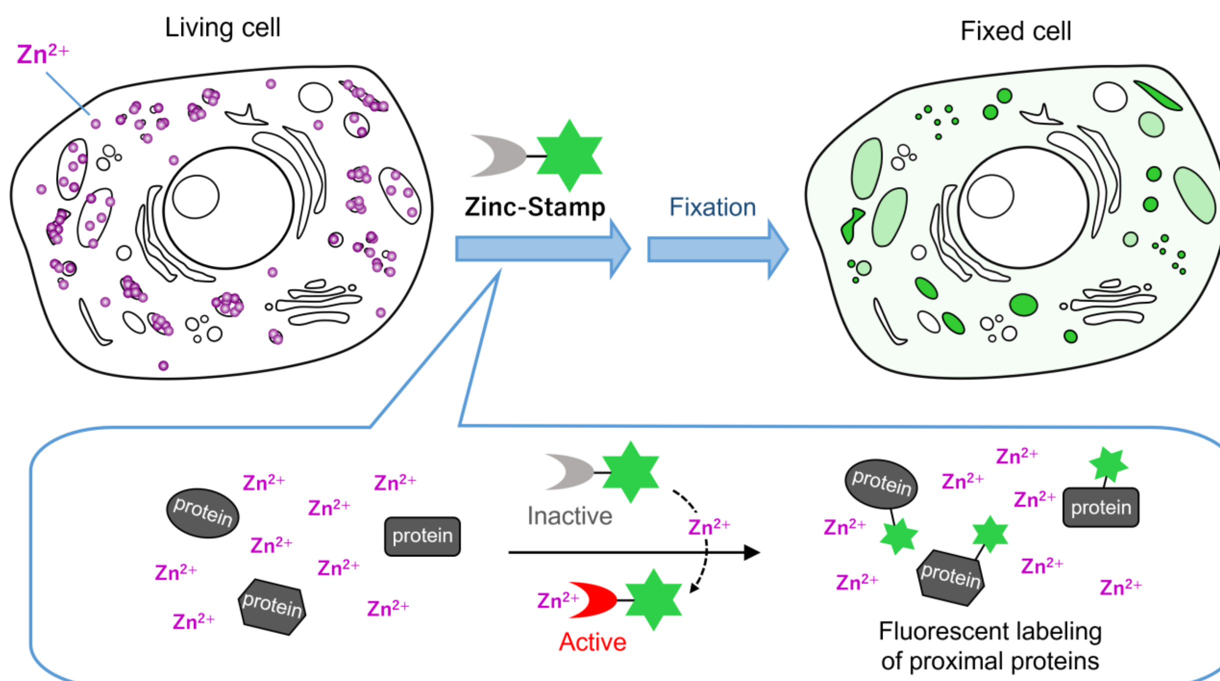
Research Use Only

Mobile Zinc-Responsive Protein Labeling Reagent

Zinc-Stamp™

80849

Zinc-Stamp™ is a chemically labeling reagent that is activated by mobile zinc ion (Zn^{2+}) and thereby covalently attaches a fluorescein tag to proximal proteins. After treatment of cells with Zinc-Stamp™ followed by cell fixation, existence and localization of Zn^{2+} can be analyzed by fluorescent microscopy. In addition, Zinc-Stamp™ enables to analyze and identify proteins involved in Zn^{2+} dynamics and transport.



Features

- Specific for Zn^{2+}. Not reactive with Mn^{2+}, Fe^{2+}, Fe^{3+}, Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}
- High S/N ratio
- Low cytotoxicity
- High cell membrane permeability - Just add Zinc-Stamp™ to culture medium
- Labeled proteins can be purified by anti-fluorescein antibody
- Various applications are available; Immunostaining, SDS-PAGE, WB, Proteomics analysis by MS

Product Name	Product Code	Size
Zinc-Stamp™, Mobile Zinc-Responsive Protein Labeling Reagent	FDV-0013A	25μg
	FDV-0013B	3 x 25μg

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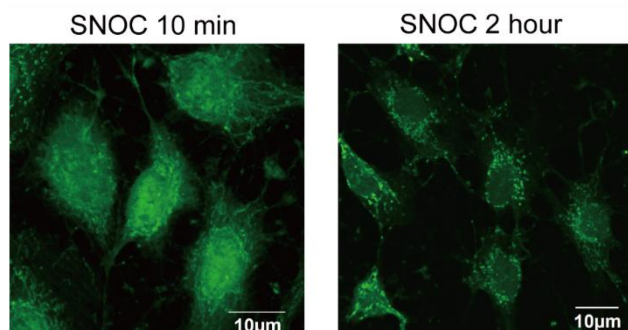
Application

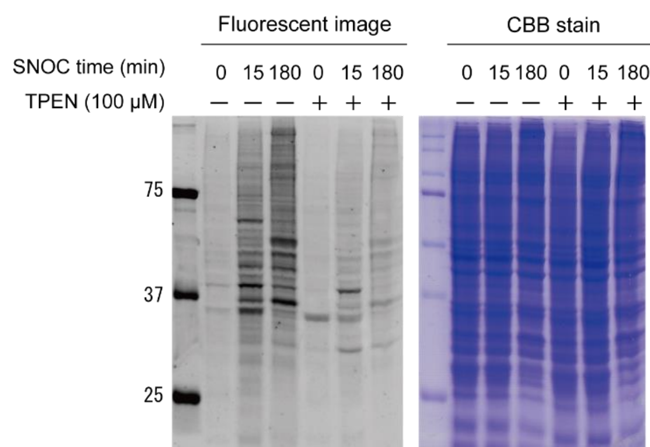
Application 1:
Imaging of dynamics and localization of mobile Zn^{2+} in cells

The localization of proteins labeled with Zinc-Stamp™ can be visualized by fixing cells. Co-staining with antibodies or organelle-markers can be performed, which enable to analyze the relationships between mobile Zn^{2+} dynamics and protein of interest, such as Zn^{2+} transport proteins.



Cells were treated with S-nitrosocysteine (SNOC; NO-generating reagent) and labeled by Zinc-Stamp™. Cells were fixed by methanol and analyzed with confocal fluorescent microscopy. The responses were observed throughout the cell after 10 minutes of oxidative stress, whereas vesicle-like fluorescent signals were observed after 2 hours of oxidative stress. This indicated that mobile Zn^{2+} was transported to vesicles to suppress toxicity.

Application 2:
Analysis of total labeled proteins

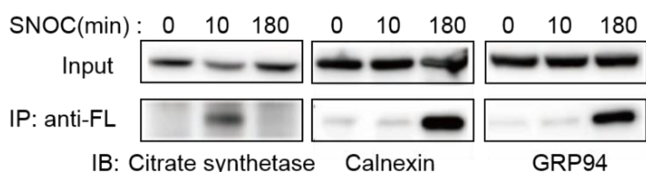


C6 glioma cells were treated with 200 μM SNOC, a NO generator, for 0, 15 and 180 min. After NO-stimulation, cells were incubated with 1 μM Zinc-Stamp™ for 10 min in the absence or presence of 400 μM TPEN, a potent Zn-chelator. Cells were lysed by SDS-sample buffer and proteins were separated in SDS-PAGE and detected by fluorescent imager for labeled proteins or CBB staining for total proteins. Band pattern of labeled proteins was dramatically changed by SNOC treatment and fluorescent signals were suppressed in the presence of TPEN.

Application 3:
Analysis of labeled proteins by WB & MS

C6 glioma cells were treated with 200 μM SNOC for 0, 10, and 180 min and incubated with 10 μM Zinc-Stamp™ for 10 min.
After lysis of cells, labeled proteins were immunopurified by fluorescein antibody (anti-FL).
Analysis were done in Western Blot (A) and Mass Spectrometer (B).

(A) Analysis of individual proteins with western blot



Isolated proteins by anti-FL were separated by SDS-PAGE and detected by western blotting with antibodies of interest (IP-blot). In this case, mitochondrial protein (citrate synthetase), ER protein (calnexin) and Golgi apparatus protein (GRP94) were analyzed. These results indicate mobile Zn^{2+} was released from metallothionein by NO treatment, transiently accumulated in mitochondria within 10 min and finally transported to ER and Golgi apparatus.

(B) Proteomics analysis with MS

NO 0 min vs 10 min — Log_2 (10min / 0min) vs Protein

NO 10 min vs 180 min — Log_2 (180min / 10min) vs Protein

Isolated proteins by anti-FL were separated by SDS-PAGE and digested by trypsin. Then, each sample was labeled with a stable isotope by the TMT method. Comparative analysis was performed using LC-MS/MS with combinations of NO treatment of 0 minutes/10 minutes and 10 minutes/180 minutes. The change in the amount of proteins labeled with Zinc-Stamp™ between samples could be comprehensively analyzed.

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