funakoshi
FRONTIERS IN LIFE SCIENCE

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Tokyo 113-0033, Japan

Zin-Pro Capture <Mobile Zinc-Responsive Protein Labeling Reagent>

Catalog No. FDV-0013A/ B

Research use only, not for human or animal therapeutic or diagnostic use.

Product Background

Zn^{2+} is tightly bound to proteins and plays essential roles in precise protein folding, and enzymatic function of metalloproteins as cofactor. A simulation suggests about 10% of proteins are estimated as Zn^{2+}-binding proteins[1]. Mobile Zn^{2+}, which is free form or loosely bound to protein surface, is the second class of zinc population and act as a signaling molecule. Recent evidences show an abnormal increase in mobile Zn^{2+} concentration induce pathological events including neuronal degeneration etc. Although fluorescent probes for real time monitoring of mobile Zn^{2+} in live cells have been developed, these probes could not reach comprehensive characterization of Zn^{2+} distribution mechanism and how Zn^{2+} mobiles in cells. To approach zinc homeostasis in cells and to identify proteins involved in zinc distribution on the molecular level, chemical reagents which can be applied for protein experiments are desirable.

Zin-Pro Capture is a chemically labeling reagent of a fluorescein tag to proximal proteins only in the presence of mobile Zn^{2+} ion (Figure 1). Zin-Pro Capture has high selectivity for Zn^{2+} ion, not showing labeling activity with the other abundant metal ions such as Mn^{2+}, Fe^{2+}/Fe^{3+}, Ca^{2+} and Mg^{2+}. Its Zn^{2+}-responsive labeling activity enable to monitor mobile Zn^{2+} species in the cell on the molecular basis. After labeling Zn^{2+}-proximal proteins with fluorescein tag in living cells by Zin-Pro Capture, cells are lysed and the labeled proteins are subsequently enriched by immunoprecipitation with anti-fluorescein antibody. The labeled and purified proteins are characterized by western blotting with antibodies against protein of interest or comprehensively identified by proteomics mass analysis. Observation of intracellular localization of labeled proteins by Zin-Pro Capture is also available in the fixed cells. Miki *el al.* showed that mobile Zn^{2+} in C6 glioma cells was dramatically released to cytosol from metal binding proteins such as metallothionein by oxidative stress and subsequently accumulated in vesicles[2]. This study revealed with Zin-Pro Capture (AIZin2 in the paper[2]) that oxidative stress induced Zn-rich vesicles in glioma cells were identified as COPII, COPI and ER-Golgi intermediate compartment (ERGIC) between ER and Golgi appratus[2-3].

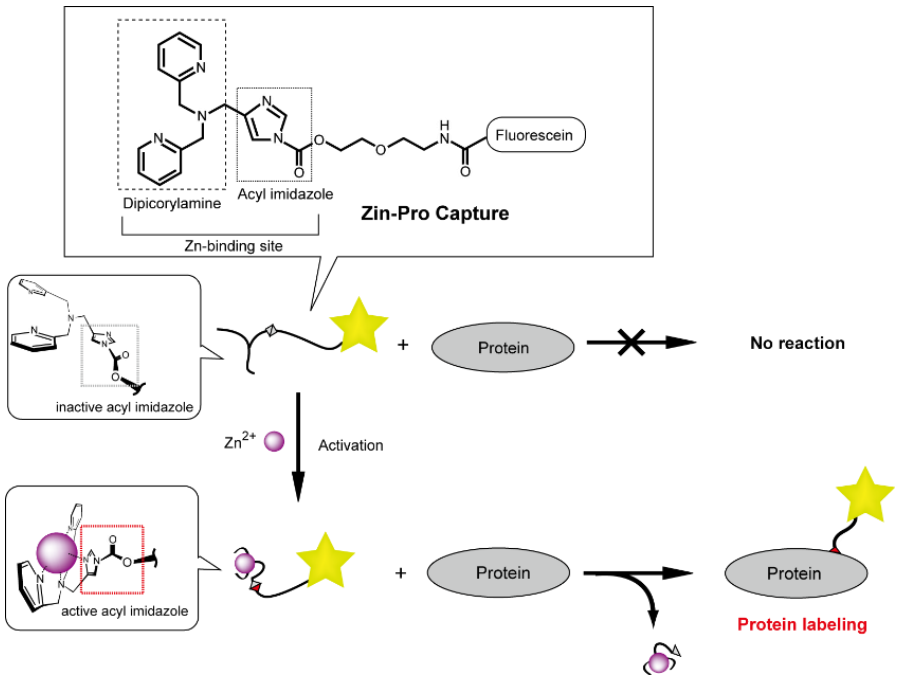


Figure 1. Principle of zinc-responsive protein labeling reagent, Zin-Pro Capture

In the absence of Zn^{2+} ion, protein labeling activity of Zin-Pro Capture is poor. On the other hand, in the presence of Zn^{2+}, Zin-Pro Capture is activated by mobile Zn^{2+} and shows protein labeling activity of fluorescent tag to proximal proteins.

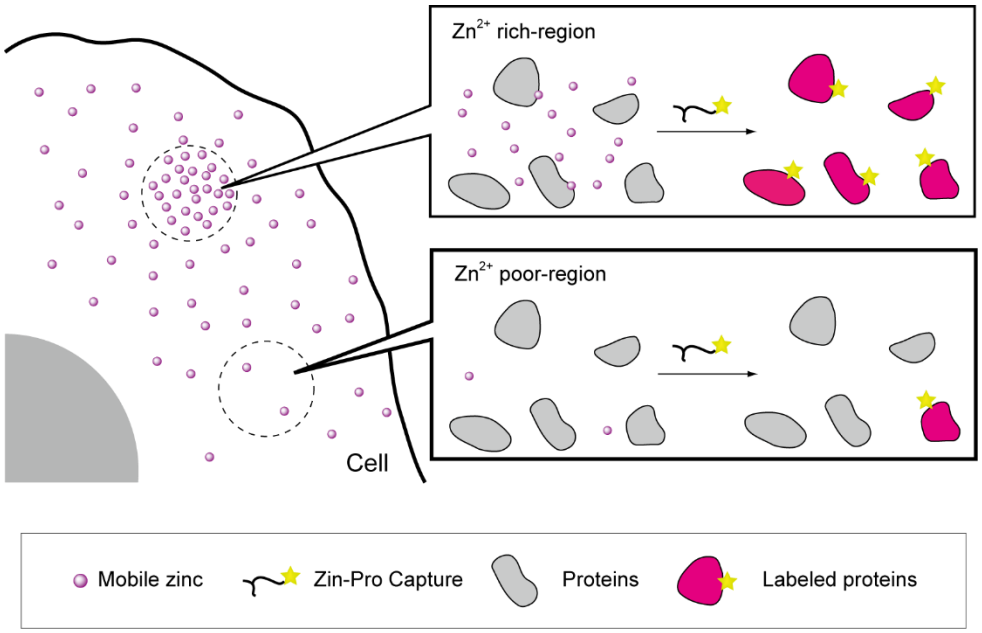
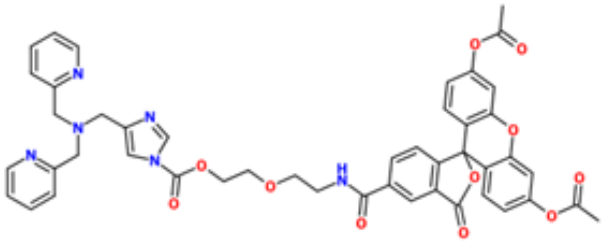


Figure 2. Overview of intracellular labeling by zinc-responsive reagent Zin-Pro Capture

Chemical labeling activity of Zin-Pro Capture is depend on the amount of Zn^{2+} ion in the cell. Highly concentrated Zn^{2+} increases Zin-Pro Capture activity and proteins located on Zn^{2+} rich compartment are highly labeled. On the other hand, in the Zn^{2+} poor region Zin-Pro Capture shows labeling activity with low efficiency and little proteins could be labeled. The labeled proteins can be purified by immunoprecipitation with anti-fluorescein antibody and applied for western blotting and mass spectrometry etc and also observed by microscopy in the fixed cells.

Description



Catalog Number: FDV-0013A/B

Size : 25 µg (FDV-0013A), 25 µg x 3 (FDV-0013B)

Formulation : $C_{46}H_{40}N_6O_{11}$

Molecular weight : 852.85 g/mol

Chemical structure : See right figure

Solubility : Soluble in DMSO

Warning: Research use only. Not for use in humans.

Fluorescent characteristics: Excitation/ Emission: 495/515 nm

*Compatible with FITC filter

Note: This reagent bears diacetylfluorescein (FDA) which shows little fluorescence emission. FDA promotes cell-permeability of the reagent. FDA is cleaved by intracellular esterases and converted to fluorescein with strong green fluorescence emission.

Application

- SDS-PAGE with fluorescent detection
- Western blotting with antibodies of interest following immunoprecipitation with anti-fluorescein antibody
- Proteomics by MS spectrometry (LC-MS/MS) following immunoprecipitation with anti-fluorescein antibody
- Immunocytochemistry with anti-fluorescein

Reconstitution and Storage

Reconstitution: Stock solution recommended concentration 0.1-1 mM in 100% DMSO.

Storage (powder): Store powder at -20°C.

Storage (solution): After reconstitution in DMSO, aliquot and store at -20°C.
Avoid repeated freeze-thaw cycles and protect from light.

How to use

General procedure for enrichment of labeled proteins

1. Prepare 1 µM of Zin-Pro Capture in the medium and replace medium just before use
2. Culture cells for 20 min with Zin-Pro Capture-containing medium
3. Remove the medium and wash cells with PBS twice
4. Add proper volume of lysis buffer and resuspended lysate well
5. After centrifugation, the supernatants is applied to desalting tools such as dialysis tube, desalting column etc. to remove extra Zin-Pro Capture reagents and their hydrolyzed products.
6. After desalting, add proper amount of anti-fluorescein antibody to lysate and incubate at 4°C
7. Add proper amount of protein A/G agarose beads and incubate at 4°C
8. Wash beads by proper buffers several times
9. Elute the labeled proteins by 50 mM Glycine-HCl buffer etc.

NOTE: Empirically optimize and determine the concentration, incubation time of Zin-Pro Capture and protocol of immunoprecipitation for your experiments.

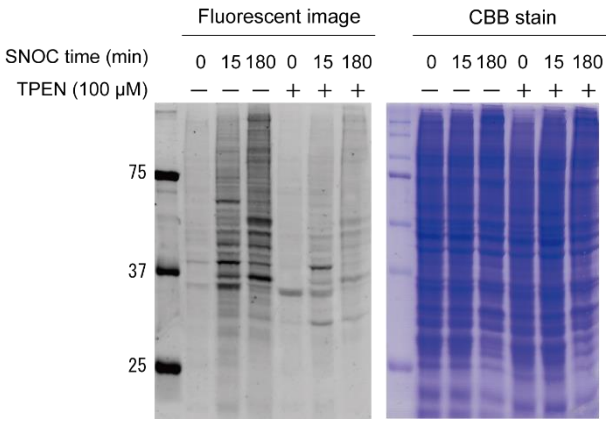
General procedure for immunocytochemistry

1. Prepare 1 μM of Zin-Pro Capture in the medium and replace medium
2. Culture cells for 20 min with Zin-Pro Capture
3. Remove the medium and wash cells with PBS
4. Cells were fixed with ice-cold MeOH
5. Following step-4, general procedures of immunocytochemistry is available

Application data

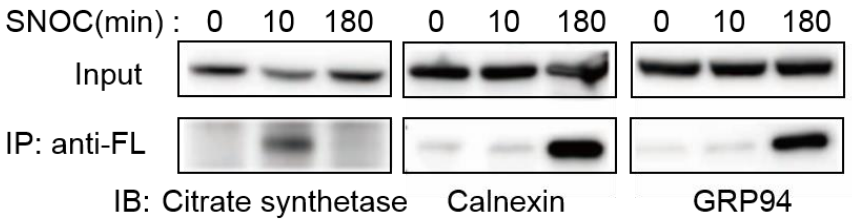
Detection of labeled proteins by SDS-PAGE

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 Zin-Pro Capture 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.



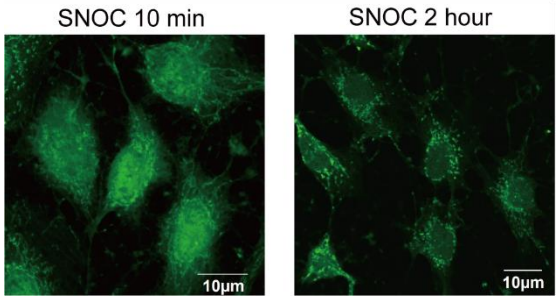
Detection of labeled proteins by IP-blot

C6 glioma cells were treated with 200 μM SNOC for 0, 10, and 180 min and incubated with 10 μM Zin-Pro Capture for 10 min. After lysis of cells, labeled proteins were immunopurified by anti-fluorescein antibody (anti-FL). 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 was released from metallothionein by NO treatment, transiently accumulated in mitochondria within 10 min and finally transported to ER and Golgi apparatus.



Observation of intracellular localization of labeled protein

C6 glioma cells were treated with 200 μM SNOC for 10 and 180 min and incubated with 10 μM Zin-Pro Capture for 10 min. After MeOH-fixation of cells, cells were observed by fluorescent microscopy with the commercial FITC filter set. Localization of labeled proteins which are proximal to high concentrated mobile zinc ion are dramatically changed by treatment time of NO.



Reference

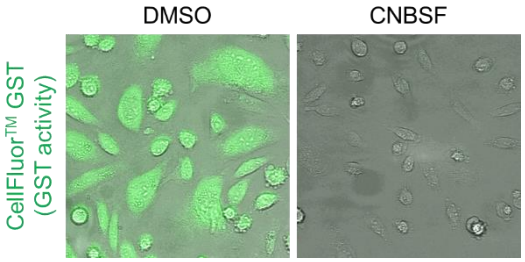
1) Andrein *et al.*, *J. Proteome Res.*, **5**, 196–201 (2006) Counting the Zinc-Proteins Encoded in the Human Genome
2) Miki *et al.*, *Nat. Methods.*, **13**, 931-937 (2016) A conditional proteomics approach to identify proteins involved in zinc homeostasis
3) Motiwala and Martin, *Nat. Methods.*, **13**, 917-918 (2016) Trail-blAIZin new directions for conditional proteomics

Related products

CellFluor™ GST <Cell-based GST Activity Assay Reagent >

CellFluor™ GST is a novel fluorescent probe for monitoring wide GST members' activity both *in celluo* or *in vitro*. CellFluor™ GST releases green fluorophore rhodamine 110 upon GST activities. This probe has cell-permeability and can detect intracellular GST activity.

Catalog No. FDV-0030
Size 0.1 µmol
Features
- Easy and quick protocol
- Broad specificity for various GST family members
- Ex/Em: 496 nm/520 nm
  (Compatible with commercial FITC filters)



CNBSF <Irreversible GST Inhibitor >

CNBSF is a novel GST inhibitor which irreversibly blocks GST enzymes. CNBSF has membrane-permeability and can be applied into live cell experiments.

Catalog No. FDV-0031
Size 10 mg
Features
- Membrane-permeable and irreversible inhibitor
- Broad specificity for various GST family members
- Covalent inhibition of GSTP1 was experimentally confirmed by MS analysis

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