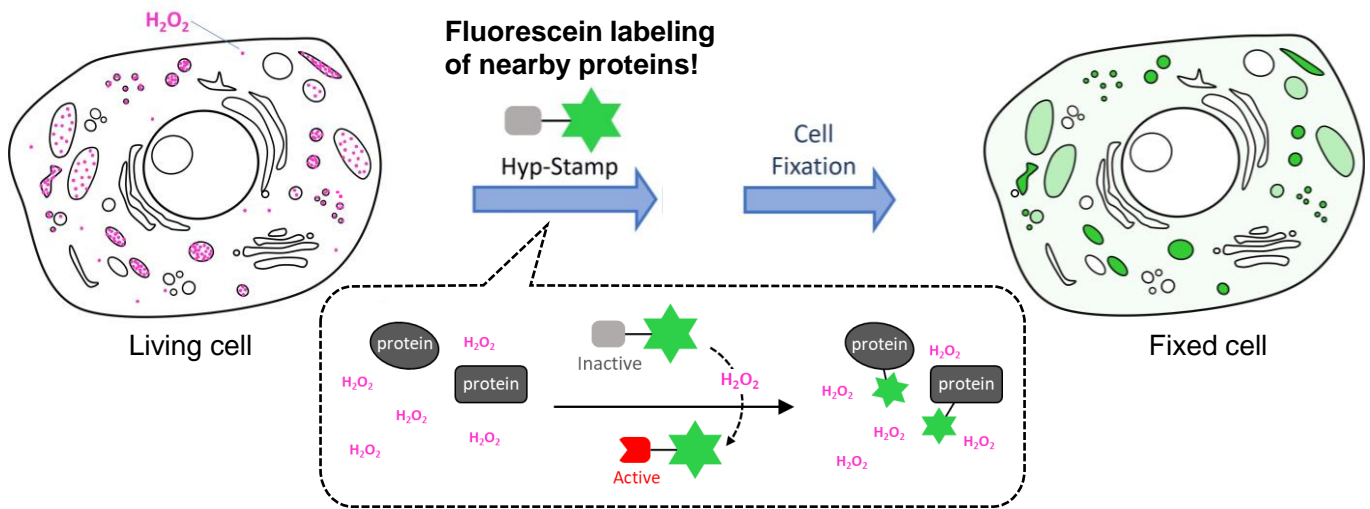


H₂O₂-Responsive Protein Labeling Reagent Hyp-Stamp™

95032

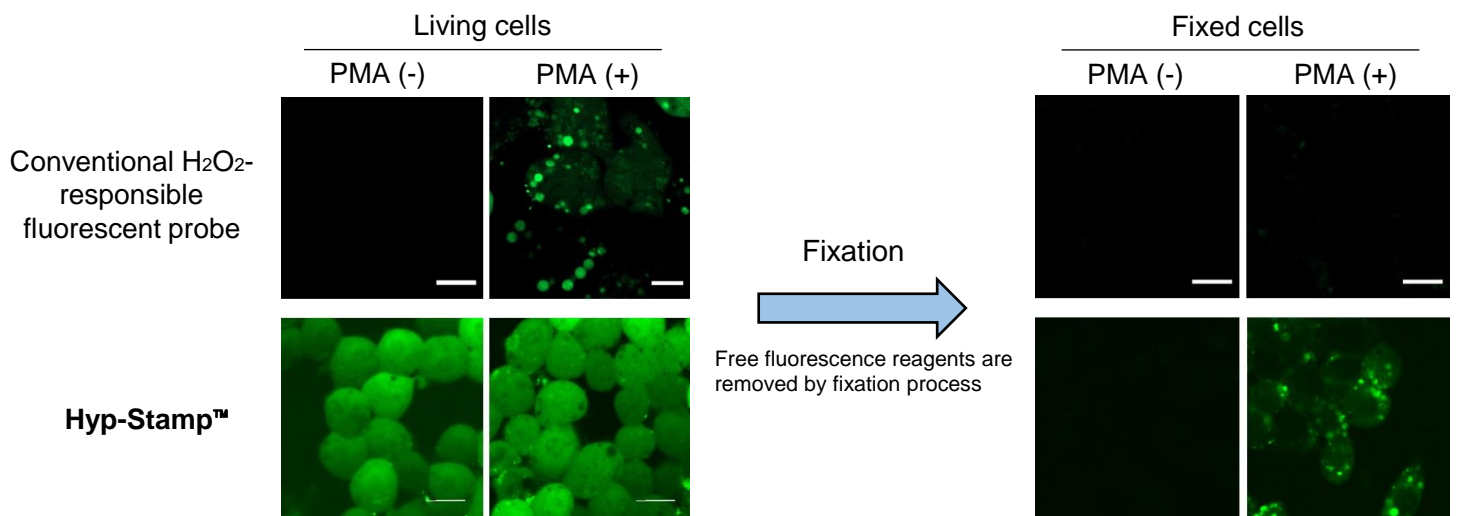


Hyp-Stamp™ is a hydrogen peroxide (H₂O₂)-responsive protein labeling reagent. It responds to cellular H₂O₂ and accomplishes fluorescein labeling of proteins localized nearby H₂O₂. Since Hyp-Stamp™-mediated fluorescein-labeled proteins remain at the original site, and after cell or tissue fixation, the H₂O₂ localization can be observed in fixed cells or tissues by fluorescent microscope. Further, Hyp-Stamp™ enables to analyze and identify individual proteins nearby H₂O₂.



Fluorescent images of RAW264.7 cells in which H₂O₂ was generated by immune stimulation

Conventional H₂O₂-responsive fluorescent probes can observe H₂O₂ generation in living cells while they are undetectable in fixed cells because they are removed in the fixation process. On the other hand, Hyp-Stamp™ can visualize the local site of H₂O₂ after fixation of the cells, while it cannot observe H₂O₂ localization in living cells.



Product Name	Product Code	Size
Hyp-Stamp, H ₂ O ₂ -Responsive Protein Labeling Reagent	FDV-0052	100 µg

Distributed by:

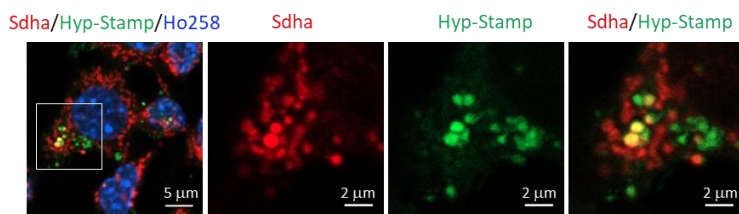
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Application

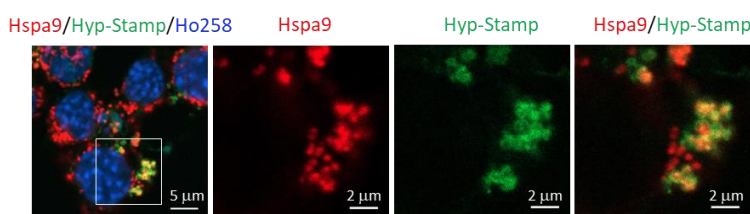
- Cell or tissue imaging after fixation of sample for observing H₂O₂ localization
- Protein analysis nearby H₂O₂ by SDS-PAGE and Western blotting
- Proteomics by MS spectrometry (LC-MS/MS) following immunoprecipitation with anti-fluorescein antibody

Experimental data

Enlarged images



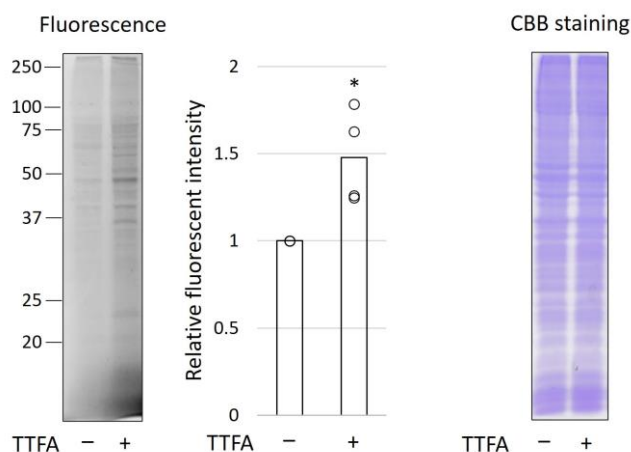
Enlarged images



Multistaining of the fluorescein-labeled proteins with antibodies in immunostimulated macrophage cells

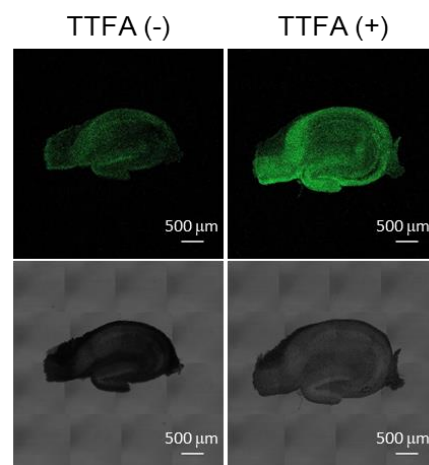
RAW264.7 cells were immunostimulated and incubated. Afterward, Hyp-Stamp™ was added, and the cells were cultured. Then, cells were fixed with cold methanol or 4% formaldehyde and immunostained with mitochondrial proteins Sdha and Hspa9. Confocal laser scanning microscope imaging shows the immunofluorescence against Sdha and Hspa9 partially merged with fluorescein-labeled vesicles.

Analysis of total fluorescein-labeled proteins under oxidative stress of mitochondria



HeLa cells were treated with mitochondrial Complex II inhibitor TTFA to induce oxidative stress, then Hyp-Stamp™ was added and incubated. After preparing a cell lysate, samples are applied to an SDS-PAGE, imaged by CBB, and in-gel fluorescence. Results revealed the amount of fluorescein-labeled protein increased by TTFA treatment, while TTFA did not affect the amount of total protein.

Imaging of the H₂O₂ localization in mouse hippocampal slices



Murine hippocampal slices were treated with mitochondrial Complex II inhibitor TTFA to induce oxidative stress, then Hyp-Stamp™ was added and incubated. The slices were fixed with 4% formaldehyde and permeabilized with cold methanol, followed by fluorescent imaging with a confocal laser scanning microscope.

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