

Hydrogen peroxide sensor HyPer

- Ratiometric measurement of intracellular hydrogen peroxide
- Direct expression in cells
- No exogenous chemical compounds or cofactors required
- High selectivity and sensitivity
- No artifactual ROS generation
- Recommended for monitoring hydrogen peroxide production inside living cells under various physiological and pathological conditions

Description

Reactive oxygen species (ROS) are tightly involved in normal cell functions as well as in development of a wide variety of pathologies. Commonly used for ROS detection, dichlorofluorescein (DCF) derivatives have several serious disadvantages: they are not specific, i.e., they are sensitive to multiple types of ROS; they cannot be targeted to specific intracellular compartments; and, most important, they can produce ROS upon light exposure, which results in artifactual ROS generation and signal amplification.

HyPer is the first fully genetically encoded fluorescent sensor capable of detecting intracellular hydrogen peroxide (H_2O_2), one of the main ROS generated by cells (Belousov *et al.*, 2006). Developed on the basis of yellow fluorescent protein inserted into the regulatory domain of *E. coli* protein OxyR (OxyR-RD) (Choi *et al.*, 2001), HyPer demonstrates submicromolar affinity to hydrogen peroxide and, at the same time, it is insensitive to other oxidants tested, such as superoxide, oxidized glutathione, nitric oxide, and peroxynitrite. HyPer does not cause artifactual ROS generation and can be used for detection of fast changes of H_2O_2 concentration in different cell compartments.

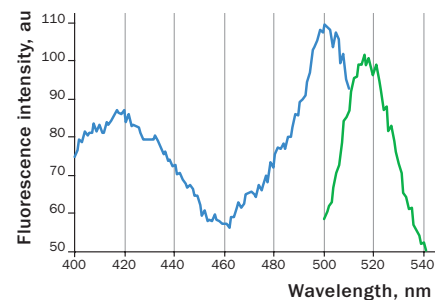
Main properties of HyPer

Characteristic	
Molecular weight	52 kDa
Polypeptide length	478 aa
Fluorescence color	green
Excitation max	420 nm and 500 nm
Emission max	516 nm
Specificity	H_2O_2
Sensitivity	submicromolar H_2O_2 concentrations
pKa	8.5
Structure	monomer
Aggregation	no
Maturation at 37°C	fast

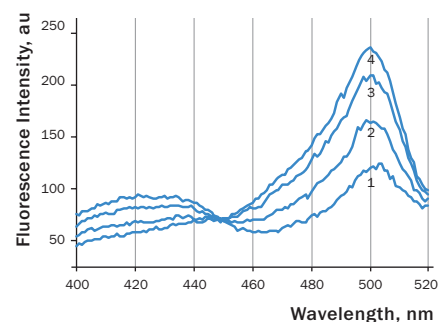
*Brightness is a product of extinction coefficient and quantum yield, divided by 1000.

Performance and use

Without H_2O_2 HyPer has two excitation peaks with maxima at 420 nm and 500 nm, and one emission peak with maximum at 516 nm. Upon exposure to H_2O_2 , the excitation peak at 420 nm decreases proportionally to the

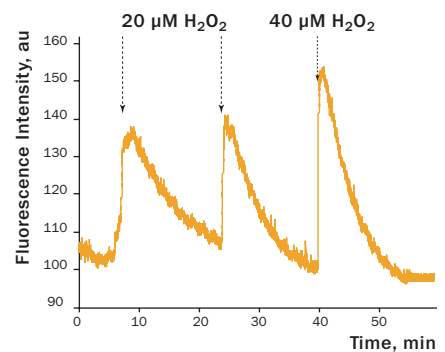


HyPer normalized excitation (green line) and emission (blue line) spectra.



Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition.

Trace 1 — without H_2O_2 ; trace 2 — 25 nM H_2O_2 ; trace 3 — 100 nM H_2O_2 ; trace 4 — 250 nM H_2O_2 . Emission was measured at 530 nm.



Kinetics of fluorescence (excitation at 490 nm, emission at 530 nm) of HyPer in *E. coli* cells suspension in the presence of 50 U/ml catalase in response to three successive additions of hydrogen peroxide.

increase in the peak at 500 nm, allowing ratiometric measurement of H₂O₂. Similarly to wild-type OxyR, oxidized HyPer is able to get reduced inside cells.

HyPer can be directly expressed by target cells individually or in fusion with a specific localization signal. It successfully folds and remains highly sensitive to hydrogen peroxide both in bacteria and in mammalian cells. If required, stable HyPer transformants can be selected using G418 (Gorman C., 1985).

HyPer suitability to generate stably transfected cells has been proven by Marinpharm company (www.marinpharm.com). Various cell lines expressing HyPer are commercially available.

HyPer can be used for monitoring and ratiometric measurement of hydrogen peroxide production in living cells under various physiological and pathological conditions.

Violet or blue excitation light should be applied for monitoring HyPer green emission changes caused by intracellular H₂O₂ production. Excitation light intensity must be individually determined for a particular biological system and instrumentation used.

Note: Yellow fluorescent core of HyPer undergoes partial photoconversion to a dark state upon irradiation with blue light. It means that an apparent "bleaching" effect occurs at the beginning of time series imaging of cells expressing HyPer protein. Unlike the real bleaching, in the case of HyPer, signal drops to the level of dynamic equilibrium between fluorescent and dark state of the chromophore, and then remains stable.

Visualization of hydrogen peroxide production in cytosol and mitochondria of HeLa cells during Apo2L/ TRAIL-induced apoptosis

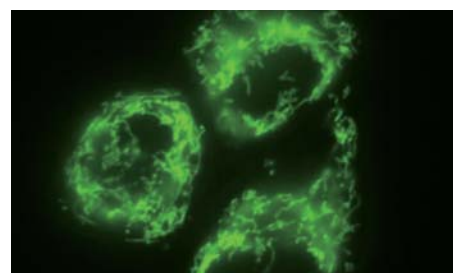
HyPer was targeted to the cytosol of HeLa cells to visualize the hydrogen peroxide production in the cells exposed to the apoptogenic protein Apo2L/TRAIL. The cells were also loaded with tetramethylrhodamine methyl ester (TMRM, 20 nM) to monitor the mitochondrial transmembrane potential.

Upon stimulation with Apo2L/TRAIL (400 ng/ml), HeLa cells degradation occurred. At 3-5 hrs after Apo2L/TRAIL addition, the cells were observed to change their shape from flat to round with plasma membrane blebs. Using simultaneous visualization of HyPer and TMRM fluorescence, we observed that cytosolic H₂O₂ started rising in parallel with a loss of the mitochondrial transmembrane potential and a change in the cell shape.

To study changes in the hydrogen peroxide level in mitochondria of HeLa cells treated with Apo2L/TRAIL, mitochondria-targeted HyPer was used. At 1-2 hrs after Apo2L/TRAIL addition, the transmembrane potential of some mitochondria started to oscillate. Simultaneous visualization of HyPer and TMRM fluorescence reveals rising of the H₂O₂ level during depolarization and decrease of the H₂O₂ level during repolarization of the mitochondria.

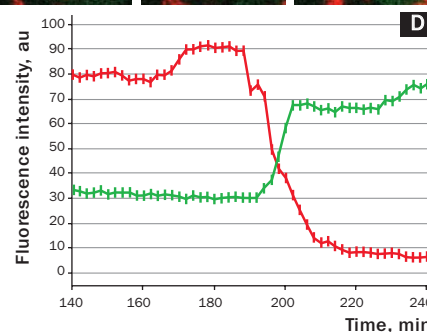
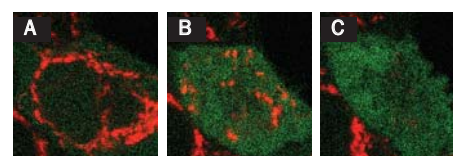
Hydrogen peroxide detection during physiological stimulation

To demonstrate HyPer suitability for detecting low concentrations of H₂O₂ generated upon physiological stimulation, PC-12 cells which expressed HyPer in the cytosol were treated with the nerve growth factor (NGF). H₂O₂ level in the cytosol of stimulated cells was monitored under the same visualization conditions as in the experiment above, but with a higher scanning rate (1 frame per 3 sec). Two patterns of cellular response were observed for 22 cells in 4 particular experiments. In most cells, H₂O₂ level started to increase almost immediately after growth factor addition and reached maximum in 3-7 min with the following decrease to the initial level in 10-20 min. Some cells demonstrated biphasic kinetics of hydrogen peroxide production. In such cells, slight initial transient H₂O₂ level increase was followed by the second higher and rapid increase in H₂O₂ production; then HyPer fluorescence gradually decreased to the initial level.



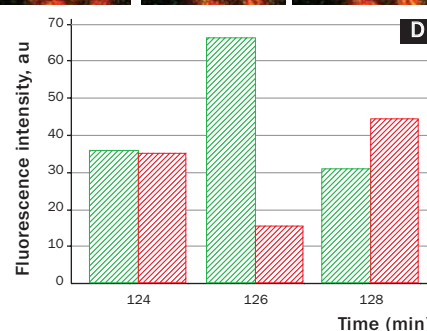
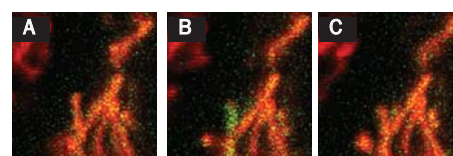
Stably transfected HeLa cells expressing mitochondria-targeted HyPer.

Image from Dr. Christian Petzelt (Marinpharm).



Dynamics of intracellular H₂O₂ production in a HeLa cell undergoing Apo2L/TRAIL-induced apoptosis.

A-C — confocal images of HeLa cells expressing cytosolic HyPer in 176 min (A), 200 min (B) and 240 min (C) after Apo2L/TRAIL addition; D — Intensities of HyPer (green) and TMRM (red) fluorescence in the cell.



Increase in the H₂O₂ level in temporarily depolarized mitochondrion during Apo2L / TRAIL -induced apoptosis.

A-C — Three sequential confocal images collected from a region of interest in a single HeLa cell; D — intensity of HyPer (green) and TMRM (red) fluorescence in a single mitochondrion in 124, 126, and 128 min after Apo2L/TRAIL addition.

Available variants and fusions

HyPer comprises fluorescent part which codon usage is optimized for high expression in mammalian cells (Haas *et al.*, 1996) and OxyR part from *E. coli* with autogenic codon usage. Effective expression of HyPer occurs in both bacteria and mammalian cells.

HyPer-Cyto variant is localized in cell cytosol.

HyPer-dMito fusion: Mitochondrial targeting sequence (MTS) was derived from subunit VIII precursor of human cytochrome C oxidase (Rizzuto *et al.*, 1989; Rizzuto *et al.*, 1995). Two MTS were fused to HyPer N-terminus. When expressed in mammalian cells, this variant is localized in mitochondria.

Recommended filter sets

Recommended Omega Optical filter sets for HyPer are QMAX-Green, XF100-2, and XF100-3. It can also be detected using Chroma Technology corporation filter set 41001 FITC/ RSGFP/ Bodipy/ Fluo 3/ DiO or the similar.

References

- Belousov *et al.* (2006) *Nat Meth.* 3(4): 281-286.
Choi *et al.* (2001) *Cell* 105: 103-113.
Gorman C. (1985) In *DNA cloning: A Practical Approach*, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), 143-190.
Haas *et al.* (1996) *Curr. Biol.* 6: 315–324.
Rizzuto *et al.* (1989) *J. Biol. Chem.* 264: 10595–10600.
Rizzuto *et al.* (1995) *Curr. Biol.* 5: 635–642.

HyPer-related products

Product	Cat.#	Description	Size
HyPer expression/source vectors			
pHyPer-Cyto	FP941	Mammalian expression vector comprising HyPer gene and allowing HyPer expression in cytosol	20 µg
pHyPer-dMito	FP942	Mammalian expression vector encoding of mitochondria-targeted HyPer	20 µg

Please contact your local distributor for exact prices and delivery information.

Third party products: stably transfected cell lines expressing HyPer

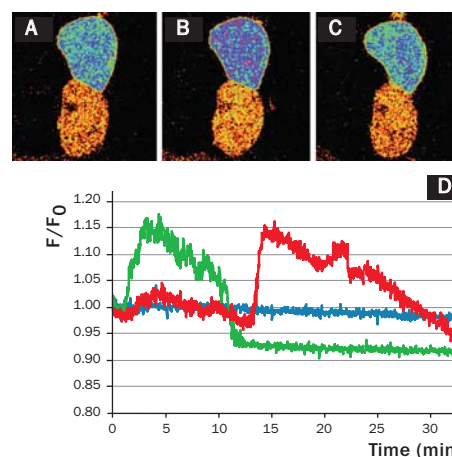
Cell line	Source	Description
HyPer-H ₂ O ₂ -sensor	human	HeLa human cervical carcinoma cells expressing HyPer in mitochondria

Cell lines are manufactured by Marinpharm GmbH (Berlin, Germany, www.marinpharm.com) under the Evrogen license.

Notice to Purchaser:

The HyPer-related material (also referred to as "Product") is intended for research use only. This product is covered by Evrogen Patents and/or Patent applications pending. Some elements of this material may be covered by third party patents issued and applicable in certain countries. No license under these patents is conveyed expressly or by implication to the recipient of the material. Users of this material may be required to obtain a patent license depending upon the particular application and country in which the material is received or used.

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Dynamics of intracellular production of hydrogen peroxide in PC-12 cells stimulated with 100 ng/ml NGF.

A-C — Pseudocolored images of cells expressing the cytosolic form of HyPer in 2 min (A), 15 min (B), and 30 min (C) after NGF addition; D — typical timecourses of HyPer fluorescence in cells after NGF stimulation (green and red lines) and in untreated cells (blue line).