Hydrogen peroxide sensor HyPer

- Ratiometric detection of intracellular H_2O_2 level changes
- High selectivity and sensitivity, no artifactual ROS generation
- Direct expression in cells, easy targeting to various subcellular compartments
- No exogenous chemical compounds required
- Recommended for monitoring H_2O_2 production inside living cells

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 importantly, 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 is insensitive to other oxidants tested, such as superoxide, oxidized glutathione, nitric oxide, and peroxinitrite. 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 under various physiological and pathological conditions.

Main properties of HyPer

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

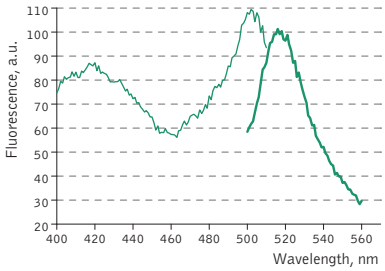
Performance and use

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 increase in the peak at 500 nm, allowing ratiometric measurement of H_2O_2. Similarly to wild-type OxyR, oxidized HyPer can be reduced inside cells.

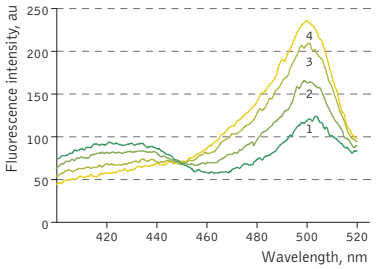
HyPer can be directly expressed by target cells individually or in fusion with a specific localization signal. It successfully folds and remains highly sensitive to hydrogen peroxide both in bacteria and in mammalian cells. If required, stable HyPer transformants can be selected using G418 [Gorman 1985]. HyPer suitability to generate stably transfected cells has been proven by Marinpharm company. 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.

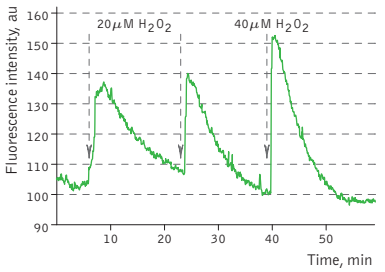
Violet and blue excitation light should be applied for monitoring HyPer green emission changes caused by intracellular H_2O_2 production. Excitation light intensity must be individually determined for a particular biological system and instrumentation used.



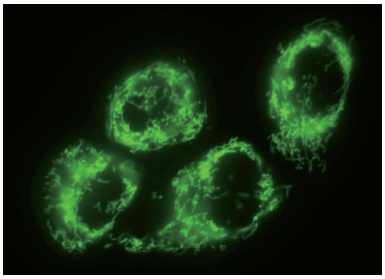
HyPer excitation (thin line) and emission (thick line) spectra.
Complete HyPer spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com



Changes in the excitation spectrum of isolated HyPer in response to H_2O_2 addition.
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* cell suspension in the presence of 50 U/ml catalase in response to three successive additions of hydrogen peroxide.



Stably transfected HeLa cells expressing mitochondria-targeted HyPer. Image was kindly provided by Dr. Christian Petzelt (Marinpharm).

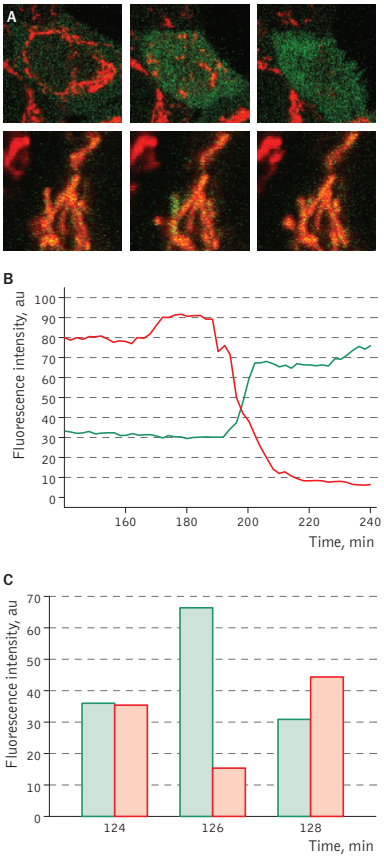
Note: Yellow fluorescent core of HyPer undergoes partial photoconversion to a dark state upon irradiation with blue light. It means that an apparent "bleaching" effect occurs at the beginning of time series imaging of cells expressing HyPer protein. Unlike the real bleaching, in the case of HyPer, signal 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 cytoplasm 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 H_2O_2 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 H_2O_2 level during depolarization and decrease of the H_2O_2 level during repolarization of the mitochondria.

Hydrogen peroxide detection during physiological stimulation: To demonstrate HyPer suitability for detecting low concentrations of H_2O_2 generated upon physiological stimulation, PC-12 cells which expressed HyPer in the cytoplasm were treated with the nerve growth factor (NGF). H_2O_2 level in the cytoplasm 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 seconds). Two patterns of cellular response were observed for 22 cells in 4 particular experiments. In most cells, H_2O_2 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 H_2O_2 level increase was followed by the second higher and rapid increase in H_2O_2 production; then HyPer fluorescence gradually decreased to the initial level.



Dynamics of H_2O_2 production in a HeLa cell undergoing Apo2L/TRAIL-induced apoptosis.
(A) Confocal images of HeLa cells expressing HyPer: upper line (from left to right) - cytosolic HyPer in 176 min, 200 min and 240 min after Apo2L/TRAIL addition; lower line (from left to right) - mitochondria-targeted HyPer in sequential images collected from a region of interest in a single HeLa cell. (B) Intensities of HyPer (green) and TMRM (red) fluorescence in the cell expressing cytosolic HyPer. (C) Intensity of mitochondria-targeted HyPer (green) and TMRM (red) fluorescence in a single mitochondrion in 124, 126, and 128 min after Apo2L/TRAIL addition.

Recommended filter sets and antibodies

HyPer can be recognized using Anti-GFP antibody (Cat.# AB011) and Anti-Tag(CGY)FP antibody (Cat.# AB121) available from Evrogen.

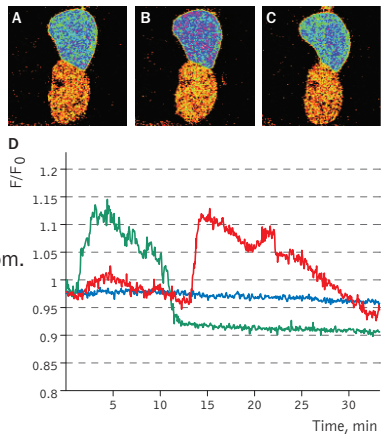
Recommended Omega Optical filter sets for HyPer are QMAX-Green, XF100-2, and XF100-3. It can also be detected using Chroma Technology Corp. filter set 41001 FITC/ RSGFP/ Bodipy/ Fluo 3/ DiO or the similar.

Available variants and fusions

HyPer mammalian expression vectors contain HyPer coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas, Park, and Seed 1996]. Humanized HyPer can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.

HyPer-AS codon usage is optimized for expression in *Arabidopsis* and *Saccharomyces*.

The available vectors encoding HyPer variants and fusions are listed below in the section HyPer-related products. For most updated product information, please visit Evrogen website www.evrogen.com. If you need HyPer codon variant or fusion construct that is not listed on our website, please contact us at product@evrogen.com.



Dynamics of intracellular production of H_2O_2 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).

Licensing opportunities

Evrogen technology embodied in HyPer is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market driven license options are offered for upgrade and novel development of products and applications. For licensing information, please contact Evrogen at license@evrogen.com.

References

Belousov, V.V. et al. (2006). Nat Methods, 3 (4): 281–286 / pmid: 16554833

Choi, H. et al. (2001). Cell, 105 (1): 103–113 / pmid: 11301006

Gorman, C. (1985). In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.), pp. 143–190.

Haas, J., E. C. Park, and B. Seed (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

HyPer-related products

Product	Cat.#	Description	Size
HyPer expression/source vectors			
pHyPer-cyto	FP941	Mammalian expression vector allowing HyPer expression in cytosol under the control of CMV promoter	20 μg
pHyPer-dMito	FP942	Mammalian expression vector encoding mitochondria-targeted HyPer	20 μg
Gateway® HyPer-AS	FP943	Gateway® entry clone for transfer of HyPer into Gateway® destination vectors; HyPer codon usage is optimized for expression in *Arabidopsis* and *Saccharomyces*	20 μg
pHyPer-nuc	FP944	Mammalian expression vector encoding nuclear-targeted HyPer	20 μg
Antibodies against HyPer			
Anti-GFP	AB011	Rabbit polyclonal antibody against EGFP, TagCFP, TagGFP, TagGFP2, TagYFP, PS-CFP2, AceGFP1, Case12 and HyPer	100 μg
Anti-Tag(CGY)FP	AB121	Rabbit polyclonal antibody against TagCFP, TagGFP, TagGFP2, TagYFP, PS-CFP2, Case12, HyPer, and EGFP	100 μg

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