



**Hydrogen Peroxide (H₂O₂)
Colorimetric Assay kit
(96 Tests)**

Zellbio GmbH (Germany)

CAT No. ZX-44106-96

www.zellx.de

Sample Types Validated for:

Fresh Urine, Buffers and Tissue Culture Medium

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Introduction

Background

Superoxide anion (O_2^-) is produced in biological systems by incomplete reduction of O_2 during respiration, which is spontaneously or enzymatically dismutated by superoxide dismutase to hydrogen peroxide (H_2O_2). Many cells produce low levels of O_2^- and H_2O_2 in response to a variety of extracellular stimuli, such as cytokines (TGF- β 1, TNF- α , and various interleukins), peptide growth factors (PDGF; EGF, VEGF, bFGF, and insulin), the agonists of heterotrimeric G protein-coupled receptors (GPCR) such as angiotensin II, thrombin, lysophosphatidic acid, sphingosine 1-phosphate, histamine, and bradykinin, and by shear stress. The addition of exogenous H_2O_2 or the intracellular production in response to receptor stimulation affects the function of various proteins, including protein kinases, protein phosphatases, transcription factors, phospholipases, ion channels, and G proteins. H_2O_2 and O_2^- may participate in the production of singlet oxygen and peroxyxynitrite which may concurrent with reactions involving iron, and under some circumstances they might play an important role in H_2O_2 toxicity.

A substantial portion of H_2O_2 lethality involves DNA damage by oxidants generated from iron-mediated Fenton reactions. Damage by Fenton oxidants occurs at the DNA bases or at the sugar residues. Sugar damage is initiated by hydrogen abstraction from one of the deoxyribose carbons, and the predominant consequence is eventual strand breakage and base release.

Assay principle

The ZellX[®] Hydrogen Peroxide (H_2O_2) assay is designed to quantitatively measure H_2O_2 in a variety of samples. A hydrogen peroxide standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The enzyme horseradish peroxidase (HRP) reacts with the substrate in the presence of hydrogen peroxide to convert the colorless substrate into a colored product. The colored product can be measured at 560 nm. Increasing levels of H_2O_2 concentration leads to linear increase in color intensity.

General information

Materials supplied in the Kit

Component	Quantity
Hydrogen Peroxide Standard (1000 μM)	110 μ L
Assay Buffer Concentrate (5 X)	12.5 mL
Substrate	2.5 mL
HRP Concentrate (50 X)	60 μ L
Clear Half Area 96 Well Plate	1 plate

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH₂O)

Microplate/ELISA reader capable of reading optical absorption at 560 nm (540-580 nm is acceptable)

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The H₂O₂ in supplied hydrogen peroxide solution is much diluted.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Reagent preparation

- i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH₂O (1 part Assay Buffer Conc. with 4 parts diH₂O), and mix well. Assay Buffer can be stored at 4°C for 3 month.
- ii. **HRP Reagent:** Vortex the suspension of HRP Concentrate prior to pipetting and then 50 µL of HRP Concentrate to 2.45 mL of Assay Buffer and mix well. HRP Reagent can be stored for 1 day.

Sample preparation

After collecting the sample, experiment should be conducted immediately, otherwise, keep the sample at -70 or -80°C. Avoid repeated freeze-thaw cycles.

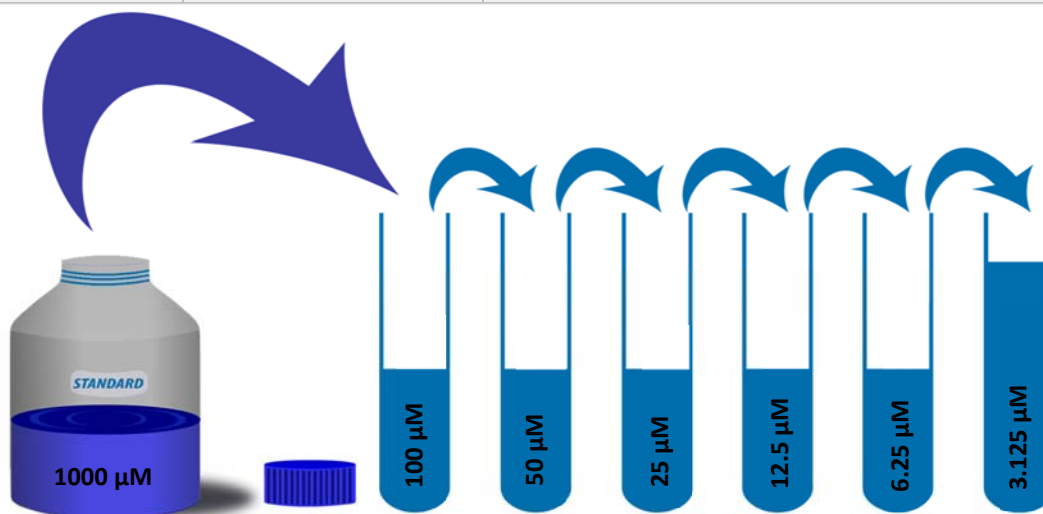
Dilute samples $\geq 1:10$ with Assay Buffer before conducting the assay.

All samples and standards must be used within 2 hours of dilution.

Standard preparation

- Prepare a 1:10 dilution of Hydrogen Peroxide Standard with Assay Buffer (mix 50 μL of standard with 450 μL of Assay Buffer), and label as the Standard No.6 (100 μM).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 μM standard.

<i>No.</i>	<i>Concentration</i>	<i>Material needed</i>
Standard No.6	100 μM	50 μL H ₂ O ₂ Standard + 450 μL Assay Buffer
Standard No.5	50 μM	200 μL Standard No.6 + 200 μL Assay Buffer
Standard No.4	25 μM	200 μL Standard No.5 + 200 μL Assay Buffer
Standard No.3	12.5 μM	200 μL Standard No.4 + 200 μL Assay Buffer
Standard No.2	6.25 μM	200 μL Standard No.3 + 200 μL Assay Buffer
Standard No.1	3.125 μM	200 μL Standard No.2 + 200 μL Assay Buffer
Standard No.0	0 μM	200 μL Assay Buffer



All standard must be used within 2 hours of preparation

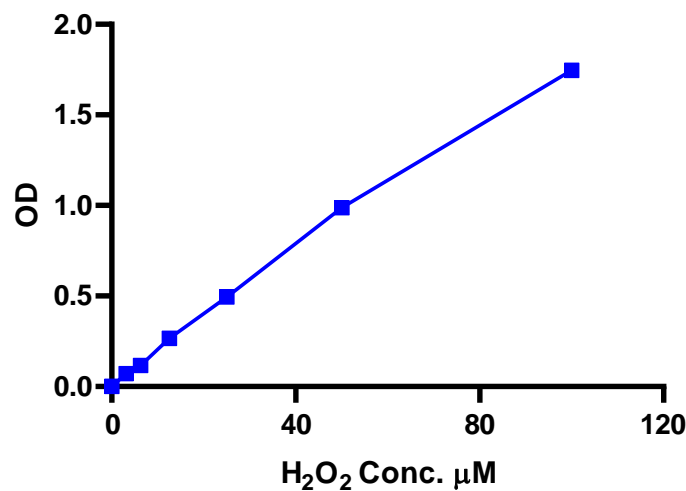
Assay Procedure

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of Assay Buffer as the Zero standard.
3. Add 25 µL of the Substrate to each well using a multichannel/repeater pipette.
4. Add 25 µL of the HRP Reagent to each well using a multichannel/repeater pipette
5. Incubate at room temperature for 15 minutes.
6. Read the optical density at 560 nm. (540-580 nm)

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs for the zero standard from all OD values
(for example if the OD value of zero standard, and standard 6 are 0.087, and 1.086 respectively; then the adjusted ODs equal 0 and 0.999 respectively.)
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 100 µM of Hydrogen Peroxide is equivalent to 3.4 µg/mL.



A typical standard curve of ZELLX[®] H₂O₂ Assay kit

Run your own standard curves for calculation of results**Assay range**

The detection limit of ZellIX[®] H₂O₂ assay was determined as 1.96 µM.

Sensitivity

The sensitivity of the ZellIX[®] H₂O₂ assay was determined as 1.83 µM.

Precision

Intra-Assay Precision (Precision within an assay): 3 buffer samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 buffer samples were tested in duplicate on 12 different assays over multiple days.

<i>Item</i>	<i>%CV</i>
Intra assay	2.1, 2.4, 5.9
Inter assay	3.7, 4.5, 4.3

Protocol summary

Add 50 μ L samples/standard into duplicate wells



Add 50 μ L Assay Buffer into duplicate wells as zero



Add 25 μ L Substrate to each well



Add 25 μ L HRP Reagent to each well



Incubate 15 min at RT



Read the absorbance at 560nm

References

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