Hydrogen Peroxide (H_2O_2)

Fluorometric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-33106-96

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 !!!

Table of Contents

Please read this insert completely prior to using the product.

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Introduction

Background

Superoxide anion (O_2^-) is produced in biological system 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-ß1, TNF-a, 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 peroxynitrite 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 (H2O2) 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 fluorescent product. The fluorescent product can be measured at 590 nm with excitation at 570 nm. Increasing levels of H_2O_2 concentration leads to linear increase in fluorescent intensity.

General information

Materials supplied in the Kit

Component	*Quantity*
Hydrogen Peroxide Standard (100 µM)	110 µL
Assay Buffer Concentrate (5 X)	12.5 mL
Fluorescent Detection Reagent	2.5 mL
HRP Concentrate (100 X)	30 µL
Black Half Area 96 Well Plate	1 plate

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Storage instruction

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

Materials required but not supplied

Deionized water (diH_2O)

Microplate reader capable of reading fluorescent at 590 nm with excitation at 570 nm

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 H_2O_2 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 diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), 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 25 µL of HRP Concentrate to 2.475 mL of Assay Buffer and mix well. HRP Reagent can be stored for 1 day.

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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 ≥ 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 (10 µM).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 µM standard.

No.	*Concentration*	*Material needed*
Standard No.7	10 µM	50 µL H_2O_2 Standard + 450 µL Assay Buffer
Standard No.6	5 µM	200 µL Standard No.7 + 200 µL Assay Buffer
Standard No.5	2.5 µM	200 µL Standard No.6 + 200 µL Assay Buffer
Standard No.4	1.25 µM	200 µL Standard No.5 + 200 µL Assay Buffer
Standard No.3	0.625 µM	200 µL Standard No.4 + 200 µL Assay Buffer
Standard No.2	0.3125 µM	200 µL Standard No.3 + 200 µL Assay Buffer
Standard No.1	0.1562 µM	200 µL Standard No.2 + 200 µL Assay Buffer
Standard No.0	0 µM	200 µL Assay Buffer

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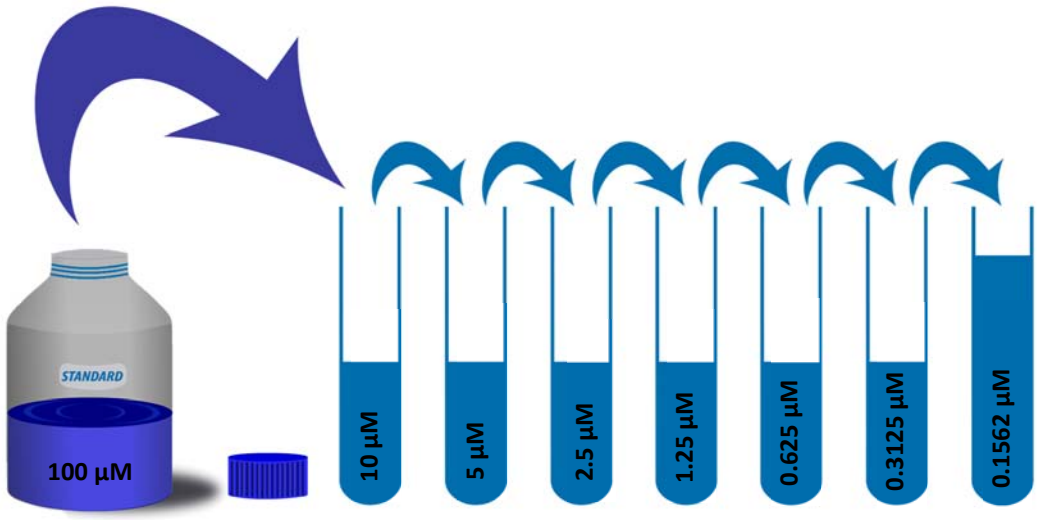

All standard must be used within 2 hours of preparation

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 Fluorescent Detection Reagent 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 fluorescent intensity at 590 nm with excitation at 570.

Calculation

- Average the duplicate Fluorescent Intensity readings for each standard and sample.
- Subtract the mean Fluorescent Intensity for the zero standard from all Fluorescent Intensity values (for example if the Fluorescent Intensity value of zero standard, and standard 6 are 0.087, and 1.086 respectively; then the adjusted Fluorescent Intensity 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 Fluorescent Intensity values.
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

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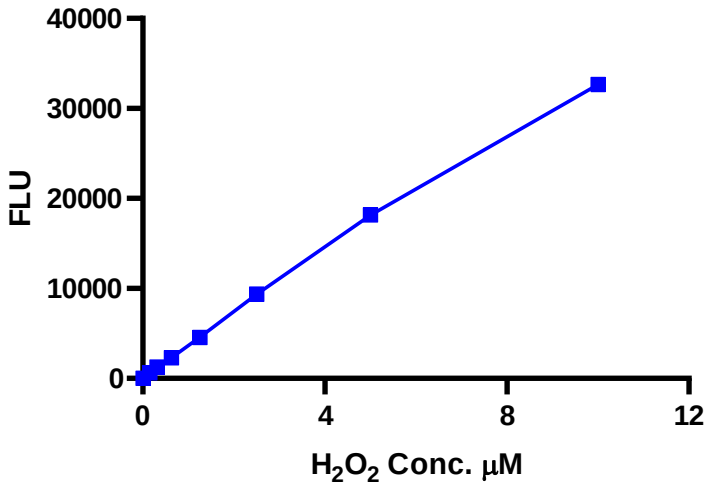
Conversion Factor: 100 nM of Hydrogen Peroxide is equivalent to 3.4 ng/mL.



A typical standard curve of ZellX® H_2O_2 Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® H_2O_2 assay was determined as 52 nM.

Sensitivity

The sensitivity of the ZellX® H_2O_2 assay was determined as 38 nM.

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 14 different assays over multiple days.

Item	*%CV*
Intra assay	3.6, 3.8, 5.7
Inter assay	12.1, 7.0, 4.3

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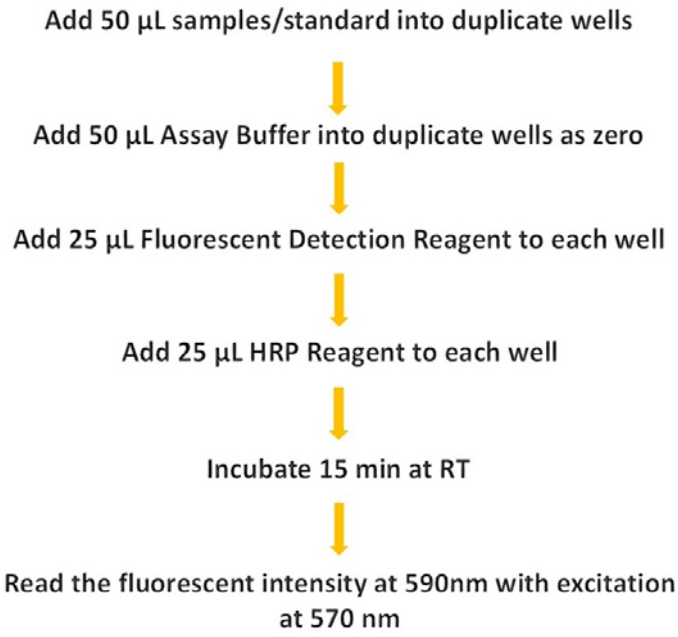
Protocol summary



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References

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