CheKine™ Micro Hydrogen Peroxide (H_2O_2) Assay Kit

Cat #: KTB1041 Size: 96 T/480 T

	Micro Hydrogen Peroxide (H_2O_2) Assay Kit		
REF	**Cat #:** KTB1041	LOT	**Lot #:** Refer to product label
	Detection range: 1 μM-100 μM		**Sensitivity:** 1 μM
	Applicable samples: Serum, Plasma, Urine, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Hydrogen peroxide (H_2O_2) is the most common reactive oxygen molecule in living organisms and is a by-product of reactive oxygen metabolism. It is mainly produced by SOD and XOD and degraded by CAT and POD. Hydrogen peroxide is not only one of the important reactive oxygen species, but also the hub of mutual conversion of reactive oxygen species. On the one hand, H_2O_2 can directly or indirectly oxidize nucleic acids, proteins and other biological macromolecules in the cell, and damage the cell membrane, thereby accelerating the senescence and disintegration of cells; on the other hand, hydrogen peroxide is also a key regulator in many oxidative emergency responses. factor. Hydrogen peroxide can activate NF-κB and other factors. These hydrogen peroxide-related signal pathways are related to many diseases such as asthma, inflammatory arthritis, arteriosclerosis, and neurodegenerative diseases. Hydrogen peroxide is also closely related to cell apoptosis and cell proliferation. CheKine™ Micro hydrogen peroxide (H_2O_2) Assay kit provides a simple and easy-to-use method for measuring H_2O_2 content in serum, plasma, cells, tissues and other biological fluids. The kit uses hydrogen peroxide to oxidize divalent iron ions to produce trivalent iron ions, and then form a purple product with xylenol orange (xylenol orange) in a specific solution. The OD value at 580 nm is proportional to the concentration of hydrogen peroxide. So as to achieve the determination of the concentration of hydrogen peroxide.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Reaction Buffer	5 mL	25 mL	-20℃, protected from light
H_2O_2 Standard (1 M)	0.1 mL	0.1 mL	-20℃, protected from light
Assay Buffer (10×)	10 mL	50 mL	4℃

Materials Required but Not Supplied

- Microplate Reader or Visible Spectrophotometer capable of measuring absorbance at 580 nm
- Incubator
- 96-well Plate with clear flat bottom or Microglass cuvette, precision Pipettes, Disposable Pipette Tips
- Centrifuge
- Deionized Water
- Dounce homogenizer (for Tissue Samples)
- 10 kDa MW Spin filter or 30% $ZnSO_4$ (for deproteinization step)

Reagent Preparation

Reaction Buffer: Ready to use as supplied. Equilibrate to room temperature and protect from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.

H_2O_2 Standard (1M): Ready to use as supplied. Equilibrate to room temperature and protect from light during the assay. Aliquot standard so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.

Assay Buffer (1×): Equilibrate to room temperature before use. Make a 1:10 dilution of the concentrated Assay Buffer (10×) with deionized water. This Assay Buffer (1×) should be used to dilute the H_2O_2 standards and samples prior to assaying. When stored at 4C°, this diluted Assay Buffer (1×) is stable for at least two months.

Standard preparation: Prepare 2 mM of H_2O_2 Standard by diluting 2 µL 1 M H_2O_2 Standard into 998 µL Assay Buffer (1×). Prepare 100 µM of H_2O_2 Standard by diluting 50 µL 2mM H_2O_2 Standard into 950 µL Assay Buffer (1×). Using 100 µM standard, prepare Standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Std.	100 µM Standard	Assay Buffer (1×)	Concentration
1	200 µL	0 µL	100 µM
2	100 µL	100 µL	50 µM
3	40 µL	160 µL	20 µM
4	20 µL	180 µL	10 µM
5	10 µL	190 µL	5 µM
6	4 µL	196 µL	2 µM
7	2 µL	198 µL	1 µM
Blank	0	200 µL	0

Note: Always prepare a fresh set of standards per use. Diluted standard solution is unstable and must be used within 4 h. If sample is cell culture supernatant, please prepare H_2O_2 Standards with culture medium.

Sample Preparation

1. Animal Tissues: Wash cells with cold PBS to remove excess blood thoroughly. Weigh about 0.1 g Tissue and add 1 mL ice-cold Assay Buffer (1×), homogenize on ice. Centrifuge at 12,000 g for 5 min at 4℃, take the supernatant for further analysis.

2. Plant Tissues: Weigh 0.1 g tissue, add 1 mL ice-cold Assay Buffer (1×) and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. For Cells or Bacteria: Collect 5×10^6 cells. Wash cells or bacteria with cold PBS, add 1 mL ice-cold Assay Buffer (1×), homogenize on ice. Centrifuge at 12,000 g for 5 min at 4℃, take the supernatant for further analysis.

4. Serum, Plasma and Urine (and other biological fluids): The protein in the sample needs to be removed first. After removing the protein, the supernatant was taken. The way to remove protein: filter with a 10 kDa Spin filter and take the filtrate for H_2O_2 assay, or mix the sample with 30% $ZnSO_4$ solution at 20:1 and vortex, then centrifuge at 10,000 g for 5 min at room temperature. Use

supernatant for H_2O_2 assay.

Note: We recommend performing several dilutions of your sample to ensure the readings are within the standard value range. We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for 1 month. The following substances interfere and should be avoided in sample preparation. Ferric salts, iron salts, sucrose, glucose, ascorbic acid, SDS (>0.2%), sodium azide.

Assay Procedure

1. Preheat the Microplate Reader or Visible Spectrophotometer for more than 30 min, and adjust the wavelength to 580 nm. Visible Spectrophotometer deionized water zero.

2. Add the following reagents to 96 well plate or Microglass cuvette:

Reagent	Standard Well (μL)	Test Well (μL)
Different concentration of Stds.	60	0
Sample	0	60
Reaction Buffer	40	40

3. Mix well and incubate 10 min at 37℃.Measure the absorbance A_{Blank}, $A_{Standard}$, A_{Test} at 580 nm. Calculate $\Delta A_{Standard}= A_{Standard}-A_{Blank}$, $\Delta A_{Test}=A_{Test}-A_{Blank}$.

Note: Std. Blank is the Blank Well.

Data Analysis

1. Drawing of standard curve

With the concentration of the standard Solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis,draw the standard curve y=kx+b.

2. Calculation of H_2O_2 content

Substitute the ΔA_{Test} into the equation to obtain the y value (μM).

The concentration of H_2O_2 in sample [Hydrogen Peroxide] (μM) =y×n

Note: If the ΔA_{Test} values are higher than the $\Delta A_{Standard}$ value for the 100 μM standard, dilute sample in Assay Buffer (1×) and repeat this assay. Multiply the results by the dilution factor n.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

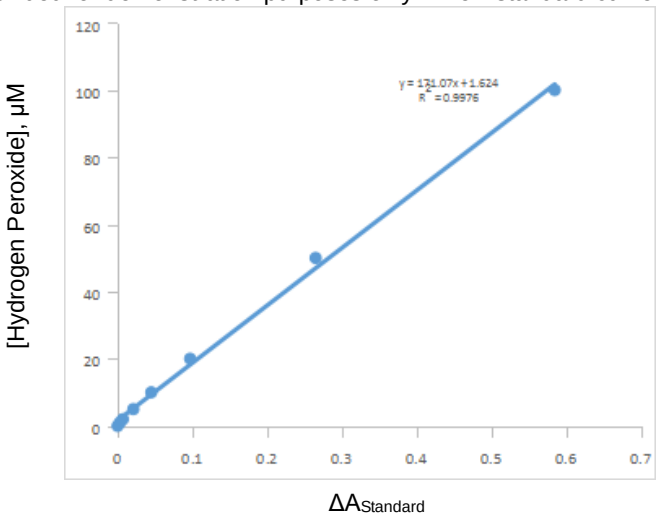

Fig. Standard Curve of H_2O_2

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.