CheKine™ Hydrogen Peroxide (H_2O_2) Assay Kit

Item NO.	Product Name
KTB1041	CheKine™ Hydrogen Peroxide (H_2O_2) Assay Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

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TABLE OF CONTENTS

INTRODUCTION

Background

Hydrogen Peroxide (H_2O_2), a reactive oxygen metabolic by-product, which serves as both an intracellular signaling messenger and a source of oxidative stress. Hydrogen peroxide is generated in cells via multiple mechanisms such as the NOX-mediated ROS production by neutrophils and macrophages (respiratory burst) or by the dismutase of superoxide anions produced as a result of electron leak during mitochondrial respiration. Functioning through NF-κB and other factors, hydroperoxide-mediated pathways have been linked to asthma, inflammatory arthritis, atherosclerosis, diabetic vasculopathy, osteoporosis, neurodegenerative diseases, Down's syndrome and immune system diseases.

Assay principle

Abbkine Hydrogen Peroxide Assay Kit provides a simple and easy colorimetric assay for measuring hydrogen peroxide in serum, plasma, cell culture supernatants, tissue/cell lysates and other biological fluids. In the assay, H_2O_2 oxidize ferrous to ferric ion, and xylenol orange binds ferric ion with high selectivity to form a colored (purple) product that can be measured by colorimetric method at OD 580 nm. The Hydrogen Peroxide present in the sample is proportional to the signal obtained.

Storage/Stability

Storage at -20°C and Keep from light. Stable for at least 12 months at recommended temperature from date of shipment.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity			Storage conditions
	48T	96T	480T	
Reaction Buffer	2.5 mL	5 mL	25 mL	-20ºC, protect from light
H_2O_2 Standard (1M)	0.1 mL	0.1 mL	0.1 mL	-20ºC, protect from light
Assay Buffer (10X)	5 mL	10 mL	50 mL	4ºC

Other supplies required, Not Supplied

- Standard microplate reader capable of measuring absorbance at 580 nm
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Assorted glassware for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (for tissue samples)
- 10 KDa MW Spin filter (for deproteinization step)

Technical hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

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°C.

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°C

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

Standard preparation

- Always prepare a fresh set of standards per use.
- Diluted standard solution is unstable and must be used within 4 hours

Prepare 2 mM of H_2O_2 Standard by diluting 2 µL H_2O_2 1 M Standard into 998 µL Assay Buffer. Prepare 20 µM of H_2O_2 Standard by diluting 10 µL 2mM H_2O_2 Standard into 990 µL Assay Buffer. Using 20 µM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

	Volume of 20 µM Standard	Assay Buffer (1x)	Concentration
Std.1	200 µL	0 µL	20 µM
Std.2	160 µL	40 µL	16 µM
Std.3	120 µL	80 µL	12 µM
Std.4	80 µL	120 µL	8 µM
Std.5	40 µL	160 µL	4 µM
Std.6	20 µL	180 µL	2 µM
Std.7	10 µL	190 µL	1 µM
Blank	0	200 µL	0

Note: If sample is cell culture supernatant, please prepare H_2O_2 Standards with culture medium.

Sample Preparation

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°C for one month.

Tissue samples: Perfuse tissue with cold PBS to remove any red blood cells. Homogenize tissue at 1 mL/0.1 g in cold Assay Buffer. Centrifuge at 12,000g for 5 minutes at 4°C. Use supernatant for H_2O_2 assay.

Cell (adherent or suspension) samples: Harvest the amount of cells needed for each assay (initial recommendation = $1x10^6$ cells/assay). Wash cells with cold PBS. Resuspend cells in 200 µL of Assay Buffer. Homogenize cells quickly by pipetting up and down a few times. Centrifuge at 12,000g for 5 minutes at 4°C. Use supernatant for H_2O_2 assay.

Collect cell culture supernatant, serum, plasma, urine and other biological fluids (contains 0.8 - 6 µM H_2O_2). Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Remove particulate pellet.

Samples, such as cell lysate, tissue lysate or plasma should be filtered through a 10 KDa MW spin filter to remove all proteins and then kept at -80°C for storage.

Assay procedure

1. Add 60 µL of diluted standard and sample per well.
2. Add 40 µL of Reaction Buffer per well quickly. Tap plate to mix briefly and thoroughly.
3. Incubate for 10 min at 37°C in the dark. Read optical density at 580nm.

DATA ANALYSIS

Calculation of results

Subtract blank OD from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The H_2O_2 concentration of Sample is calculated as

$$[\text{Hydrogen Peroxide}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times n \ (\mu M)$$

Note: If the OD_{SAMPLE} values are higher than the OD value for the 20 µM standard, dilute sample in Assay Buffer (1x) and repeat this assay. Multiply the results by the dilution factor.

Typical data

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