(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Hydrogen Peroxide (H_2O_2) Assay Kit

Catalog No.: BC00028

Size: 150T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Hydrogen Peroxide (H_2O_2) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, cells, serum, plasma, urine
Assay Type	Quantitative
Detection Instrument	Microplate reader (240 nm, (540-570 nm, optimal detection wavelength 560 nm))
Range	1-100μM
Sensitivity	0.7809μM

Product Introduction

Hydrogen peroxide is a byproduct of reactive oxygen species metabolism and is a key regulatory factor in many oxidative stress reactions. Hydrogen peroxide can activate factors such as NF-κB, and these hydrogen peroxide-related signaling pathways are associated with many diseases such as asthma, inflammatory arthritis, atherosclerosis, and neurodegenerative diseases. Hydrogen peroxide is also closely related to apoptosis and cell proliferation.

Product Features

- This kit is convenient and quick, usually 10-20 samples can be measured within 40-60 minutes.

Principle

This kit uses hydrogen peroxide to oxidize ferric ions to produce ferric ions, which then react with xylenol orange in a specific solution to form a purple product, thereby determining the concentration of hydrogen peroxide. This kit has been improved to detect hydrogen peroxide as low as 1 micromol/L.

Components

No.	Components	Size (150T)	Storage
Reagent 1	Color Developer	15ml	-20℃
Reagent 2	Iron Reagent	Powder	-20℃
Reagent 3	Hydrogen Peroxide Standard Solution (1M)	1ml	-20℃, protect from light.
Reagent 4	Hydrogen Peroxide Detection Lysis Buffer	50ml	-20℃
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20°C for 6 months.

Preparation

- **Sample handling**

1. Preparation of cell or tissue samples

For cultured cells, first collect the cells into a centrifuge tube, discard the supernatant, add lysis buffer at a ratio of 100-200 μl of hydrogen peroxide detection lysis buffer per 1 million cells, and then fully homogenize to break and lyse the cells. Centrifuge at 4ºC at about 12,000g for 3-5 minutes, and take the supernatant for subsequent measurement. For tissue samples, add 100-200 μl of lysis buffer per 5-10 mg of tissue and homogenize. Centrifuge at 4ºC at about 12,000g for 3-5 minutes, and take the supernatant for subsequent measurement. All the above operations must be performed at 4ºC or on ice. If the prepared cell or tissue samples are not measured immediately, they can be frozen at -20ºC.

2. Preparation of cultured cell supernatant samples

The supernatant of cultured cells can be directly used for subsequent assays.

3. Preparation of serum, plasma or urine samples

Prepare 50mM phosphate buffer, pH 6.0. Dilute the sample 50 times with 50mM phosphate buffer, pH 6.0. For example, dilute 4 microliters of sample into 196 microliters of 50mM phosphate buffer, pH 6.0. After dilution, it can be used for subsequent determinations.

- **Preparation of the kit**

1. Take out all reagents and return to room temperature before use.
2. Preparation of iron reagent (you need to prepare 0.01mol/l sulfuric acid solution): Take one tube of reagent 2 and add 15ml of 0.01mol/l sulfuric acid solution and mix thoroughly (it is recommended to first add 1ml of sulfuric acid solution to reagent 2 and fully dissolve it, then transfer it to a 15ml centrifuge tube, then add 14ml of sulfuric acid solution and mix thoroughly).
3. Preparation of color development working solution: Mix the prepared iron reagent and color developer in a volume ratio of 1:1, mix thoroughly, and use immediately.

Operation process

1. Preparation of Standard Curve Assay

(1) Calibration of Hydrogen Peroxide Standards

Since hydrogen peroxide is not very stable, the actual concentration of hydrogen peroxide must be measured for calibration before use. Dilute hydrogen peroxide with a concentration of about 1M with water 100 times to make the concentration of hydrogen peroxide about 10mM, and measure A240. A240 can be measured by any of the following methods:

a. Ordinary UV spectrophotometer method: Use a UV spectrophotometer with a cuvette holder, NanoDrop 2000C, NanoDrop One ^{c}, QuickDrop and other instruments, with a quartz cuvette. Determine the cuvette path length , which is generally 1 cm . The hydrogen peroxide concentration detected by the cuvette is closest to the actual concentration.

b. Micro-volume UV spectrophotometer method: such as NanoDrop 2000, NanoDrop One, QuickDrop, Varioskan with ultra-micro-volume detection plate μDrop Plate, etc. Determine the optical path: For NanoDrop 2000, NanoDrop One, etc., it is necessary to cancel the "automatic optical path", in which case the optical path is generally 0.1cm;

the optical path of Varioskan's ultra-micro-volume detection plate μDrop Plate is generally 0.05cm. For the specific optical path of the micro-volume UV spectrophotometer, please refer to the instrument parameters.

c. 96-well UV microplate reader method (capable of detecting 240 nm wavelength): Determine the optical path length based on the parameters of the 96-well plate, which is generally 0.552 cm for a 200-microliter sample (sample volume divided by the cross-sectional area of a single well in a 96-well plate). It is generally recommended to use a dedicated 96-well UV detection plate (such as a 96-well UV plate). If a UV detection plate is not available, a regular 96-well plate can be used, but since it is not specifically designed for UV detection, it will have a very high UV absorption signal. Therefore, it is necessary to set up wells with an equal amount of double-distilled water as a blank control (generally, 200 μl of water in such a 96-well plate has an A240 of around 3.8), and this blank control must be subtracted during calculation. When using a non-UV detection plate, due to the limited detection range of the 96-well plate reader at 240 nm, it is recommended to dilute the hydrogen peroxide to about 10 mM before measuring the concentration.

   Note: All of the above methods require setting up an equal amount of double-distilled water as a blank control, and this blank control must be subtracted during the calculation.

   Concentration calculation formula: c=A/(ε×b). Where: c is the sample concentration (in units of mol/L or M); A is the absorbance value; ε is the wavelength-dependent molar extinction coefficient (in $L\times mol^{-1}\times cm^{-1}$ or $M^{-1}\times cm^{-1}$), and the molar extinction coefficient of hydrogen peroxide is $43.6M^{-1}cm^{-1}$; b=optical path (in cm).

   Therefore: Hydrogen peroxide concentration (M) = A240/(43.6×b); that is: Hydrogen peroxide concentration (mM) = 22.94×A240/b

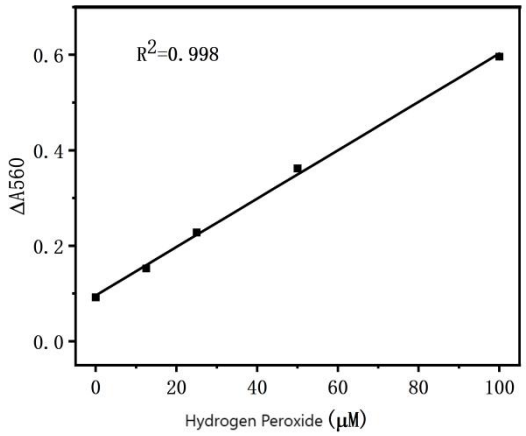
   The actual concentration of hydrogen peroxide provided by the kit is thereby calculated, and the subsequent standard curve is set according to the actual measured concentration.

   Example: After diluting the approximately 1M hydrogen peroxide provided by this kit with double-distilled water 100 times, and using a 96-well plate reader and a regular 96-well plate for detection, 200 microliters per well, three parallels per group. The average A240 of the double-distilled water control group is 3.750, and the average A240 of the hydrogen peroxide sample group is 3.974, with a difference of 0.224, and

the optical path length for 200 microliters of sample is 0.552 cm. Substituting into the formula, hydrogen peroxide concentration (mM) = 22.94×0.224/0.552=9.31, so the actual concentration of hydrogen peroxide provided by this kit is 0.931M.

(2) Setting up the standard curve

The standard should be diluted with the same solution as the sample, which can reduce the error. For example, for cell samples, the standard should be diluted with hydrogen peroxide detection lysate, and for supernatant samples of cultured cells, the standard should be diluted with the corresponding cell culture medium. The standard solution can be diluted to 100, 50, 25, 12.5, and 6.25 micromol/L by half dilution method. After knowing the concentration range of the sample after the initial determination, the standard can also be intensively determined near the sample concentration range. The figure below shows a typical graph of the standard curve determined by this kit.



2 Determination of hydrogen peroxide concentration

(1) Thaw the hydrogen peroxide assay reagent on ice or in an ice water bath.

(2) Add 50 μl of sample or standard into the test well or test tube.

(3) Add 100 μl of color development working solution to each well.

(4) Gently shake or tap to mix, and leave at room temperature (15-30°C) for 30 minutes. Then measure A560 immediately. If it is difficult to measure A560, the wavelength can be selected to be 540-570nm.

The operation table is as follows:

	Standard Tube (Well)	**Measurement Tube (Well)**

Different concentration standards (μL)	50	--
Sample to be tested (μL)	--	50
Color development working solution (μL)	100	100
Gently shake or tap to mix, and leave at room temperature (15-30°C) for 30 minutes. Then measure A560 immediately. If it is difficult to measure A560, the wavelength can be selected to be 540-570nm.		

Note: If the concentration of hydrogen peroxide in the sample is too high, it can be appropriately diluted before measurement. If the concentration of hydrogen peroxide in the sample is too low, the sample volume can be changed to 100 microliters, and the standard should also use 100 microliters, while the color working solution still uses 100 microliters. This can improve the sensitivity of the detection, but the disadvantage is that the sample needs to consume 100 microliters.

Calculation

Calculate the concentration of hydrogen peroxide in the sample based on the standard curve.

Notes

1. Some reagents that interfere with redox reactions or appear purple or close to purple under acidic conditions will interfere with the detection of hydrogen peroxide and should be avoided.
2. If the sample contains added high concentrations of iron salts, it will interfere with the measurement. However, trace amounts of iron salts contained in ordinary culture media, serum, and other samples will not interfere with the measurement.
3. A plate reader capable of measuring A560 is required for the assay (measuring 540-570nm is also possible) or a spectrophotometer capable of measuring trace samples.
4. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary

residences.