Hydrogen Peroxide (H_2O_2) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Detection instrument: Spectrophotometer / Microplate reader

Cat No: NA0612

Size: 100T/96S

Components:

Reagent I: Acetone 100mL×1. Storage at 4℃. (**Self-provided reagent**)

Reagent II: Powder×1. Storage at 4℃. Working solution: Add 3mL concentrated hydrochloric acid (37%) before use, fully dissolved. Unused reagents stored at 4°C.

Reagent III: 6mL×1. Storage at 4℃.

Reagent IV: 30mL×1. Storage at 4℃.

Standard: 1mL×1, 1mmol/mL H_2O_2 standard solution. Storage at 4℃.

Product Description:

H_2O_2 is the most common reactive oxygen molecules in organisms. It is mainly produced by the catalyzation of SOD and XOD and degraded by the catalyzation of CAT and POD. H_2O_2, which is not only one of the important reactive oxygen, but also the hub of mutual conversion of reactive oxygen. On the one hand, H_2O_2 can directly or indirectly oxidize intracellular nucleic acids, proteins and other biological macromolecules, and damage cell membranes, thus accelerating the aging and disintegration of cells. On the other hand, H_2O_2 is also a key regulatory factor in many oxidative emergency reactions.

H_2O_2 and titanium sulfate generate yellow titanium peroxide complex with the characteristic absorption at 415nm.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, transferpettor, acetone concentrated sulfuric acid (37% HCl), mortar and ice.

Procedure:

I. Sample Extraction:

1. Bacterial or cell sample: collect bacterial or cell sample to centrifuge, discard the supernatant; suggested 5 million with 1mL of regent I, splitting bacteria and cell with ultrasonication (power 20%, work time 3s, interval 10s, for 30 times); centrifuge at 8000g and 4℃ for 10min, supernatant is placed on ice for test.
2. Tissue: take 0.1g tissue add 1 ml regent I, fully grinding on ice. centrifuge at 8000g and 4℃for 10min, supernatant is placed on ice for test.
3. Serum: according to the proportion of per 100μL of serum(plasma) add 0.9mL regent I, mix well. centrifuge at 8000g and 4℃ for 10min, supernatant is placed on ice for test.

II. Determination procedure:

1 Preheat spectrophotometer/microplate reader for 30min, adjust wavelength to 415nm, set zero with distilled water.

2 Incubate Solution II, III and IV at 37℃(mammals) or 25℃ (other animals) water bath for more than 10min

3 Standard working solution: If using a 96-well plate, dilute the 1mmol/mL standard solution to 2μmol/mL standard solution with acetone, and use a trace glass colorimetric method to dilute 1mmol/mL standard solution to 1μmol/mL standard solution

4 Add reagents with the following list(reaction in EP tube):

Reagent (μL)	Test Tube (A_T)	Standard Tube (A_S)	Control Tube (A_C)
Sample	250		
Standard working solution		250	
Regent I			250
Regent II	25	25	25
Regent III	50	50	50
4000g, room temperature centrifuge for 10mins, discard supernatant.			
Regent IV	250	250	250

Add Regent IV to dissolve the precipitate (the step can remove the vegetable pigment with acetone for 3-5 times), and place it at room temperature for 5min, Transfer 200 μL to a micro glass cuvette or 96-well plate and measure the absorbance at 415 nm. The control tube need only be tested once or twice. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_C$.

III. Calculation(For Microplate reader)

A. 96-well plate

1) Cell amount

$H_2O_2(\mu mol/10^4\ cell) = \Delta A_T \div (\Delta A_S \div C) \times Vs \div (500 \times Vs \div Ve) = 0.004 \times \Delta A_T \div \Delta A_S$

2) Sample weight

$H_2O_2(\mu mol/g) = \Delta A_T \div (\Delta A_S \div C) \times V1 \div (Vs \div Ve \times W) = 2 \times \Delta A_T \div \Delta A_S \div W$

3) Protein concentration

$H_2O_2(\mu mol/mg\ prot) = \Delta A_T \div (\Delta A_S \div C) \times Vs \div (Cpr \times Vs) = 2 \times \Delta A_T \div \Delta A_S \div Cpr$

4) Serum(plasma) volume

$H_2O_2(\mu mol/mL) = \Delta A_T \div (\Delta A_S \div C) \times 10 = 20 \times \Delta A_T \div \Delta A_S$

500: cell or bacteria amount, 10^4;

C: concentration of H_2O_2 standard solution, 2μmol/mL;

Vs: sample volume, 0.25 ml;

W: Sample weight, g;

Ve: extraction volume, 1 ml;

Cpr: sample protein concentration, mg/mL;

10: serum dilution multiple. [0.1mL serum(plasma)+0.9mL regent I]÷0.1mL serum(plasma)=10.

B. **micro glass cuvette**:

Change the concentration of standard C-2μmol/mL in the above formula to C-1μmol/mL for calculation.

Note:

1. As Solution I is easily volatile, Solution I must be precooled before use. It must be ground on ice when grinding.
2. The solution in this kit is easily volatile. Please bring disposable gloves and masks.
3. If the absorbance value of the sample is greater than 1.1, it is recommended to dilute the sample with Reagent I before performing the measurement.

Experimental examples:

1. Take 0.1 g of heart and add 1 mL of Reagent I for sample processing. After centrifugation to take all the supernatant, proceed according to the determination procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T=A_T-A_C=0.083-0.046=0.039$, $\Delta A_S=A_S-A_C=0.824-0.046=0.778$. The content is calculated according to the sample mass.

   H_2O_2(μmol/g) $=2\times\Delta A_T \div \Delta A_S \div W =1$ μmol/g.
2. Take 0.1 g of tea and add 1 mL of Reagent I for sample processing. After centrifugation to take all the supernatant, proceed according to the determination procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T=A_T-A_C=0.258-0.003=0.255$, $\Delta A_S=A_S-A_C=0.637-0.003=0.634$. The content is calculated according to the sample mass.

   H_2O_2(μmol/g) $=2\times\Delta A_T \div \Delta A_S \div W =4.5$ μmol/g.

Recent Product citations：

[1] Yanan Wang,Chengzhen Liang,Zhigang Meng,et al. Leveraging Atriplex hortensis choline monooxygenase to improve chilling tolerance in cotton. Environmental and Experimental Botany. June 2019;162:364-373.(IF3.712)

[2] Xuechan Tang,Xiaoli Xie,Xin Wang,et al. The Combination of piR-823 and Eukaryotic Initiation Factor 3 B (EIF3B) Activates Hepatic Stellate Cells via Upregulating TGF-β1 in Liver Fibrogenesis. International Medical Journal of Experimental. December 2018;(IF1.420)

[3] Ying Zhao,Wengang Yu,Xiangyu Hu,et al. Physiological and transcriptomic analysis revealed the involvement of crucial factors in heat stress response of Rhododendron hainanense. Gene. June 2018;(IF2.638)

[4] Bingbing Cai,Qiang Li,Fengjiao Liu,et al. Decreasing fructose 1,6-bisphosphate aldolase activity reduces plant growth and tolerance to chilling stress in tomato seedlings. Physiologia Plantarum. December 2017;(IF3)

[5] Xiaorong Guo,Junfeng Niu,Xiaoyan Cao. Heterologous Expression of Salvia miltiorrhiza MicroRNA408 Enhances Tolerance to Salt Stress in Nicotiana benthamiana. International Journal of Molecular Sciences. December 2018;(IF4.183)

References：

[1] Satterfield C N, Bonnell A H. Interferences in titanium sulfate method for hydrogen peroxide[J].

Analytical Chemistry, 1955, 27(7): 1174-1175.
[2] Amin V M, Olson N F. Spectrophotometric determination of hydrogen peroxide in milk[J]. Journal of Dairy Science, 1967, 50(4): 461-464.
[3] Sima Y H, Yao J M, Hou Y S, et al. Variations of hydrogen peroxide and catalase expression in Bombyx eggs during diapause initiation and termination[J]. Archives of insect biochemistry and physiology, 2011, 77(2): 72-80.

Related products:

NA0870/NA0627 Malondialdehyde(MDA) Content Assay Kit
NA0789/NA0548 Xanthine Oxidase(XOD) Activity Assay Kit
NA0814/NA0572 Glucose Oxidase(GOD) Activity Assay Kit

Technical Specifications:

Limit of Detection ：0.0027 μmol/mL
Linear Range：0.0195-3 μmol/mL