Hydrogen Peroxide (H_2O_2) Content Assay Kit

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

Detection instrument: Spectrophotometer

Catalog Number: BC3590

Size: 50T/48S

Components:

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

Reagent II: Powder ×1. Storage at 4℃. Add 6mL of concentrated hydrochloric acid (37%) to dissolve fully before use. Keep the rest reagent at 4℃.

Reagent III: Liquid 12mL×1. Storage at 4℃.

Reagent IV: Liquid 60mL×1. Storage at 4℃.

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

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, table centrifuge, pipettor, 1 mL glass cuvette, acetone, concentrated hydrochloric acid (37% HCl), mortar and ice.

Sample preparation

1. H_2O_2 extraction

A. Bacteria, cells or tissue samples

The bacteria or cells were collected into a centrifuge tube, and discard the supernatant after centrifugation. Add 1mL reagent I to per 5 million bacteria or cells, and use the ultrasonic to crush the bacteria or cells (20% power, 3 seconds ultrasound, 10 seconds interval, and 30 times repetition). Centrifuge at 8000 g for 10 min with 4℃ and place the supernatant on the ice for test.

B. Tissue samples

Weigh and take about 0.1g of tissue and added with 1mL reagent I for ice bath homogenate. Centrifuge at 8000 g for 10 min with 4℃, take all that (note absorbs clean) supernatant fluid, place it on the ice for test.

C. Serum (plasma) sample: according to the proportion of per 100 μL of serum(plasma) add

0.9mL regent I, mix well. centrifuge at 8000g and 4℃ for 10min, supernatant is placed on ice for test.

Notes：

a. As Reagent I is easily volatile, Solution I must be precooled before use. It must be ground on ice when grinding.

b. The solution in this kit is easily volatile. Please bring disposable gloves and masks.

2. Procedure and Sample list

A. Preheat the spectrophotometer for more than 30min, adjust the wavelength to 415nm, and set zero with distilled water.

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

C. Dilute 1mmol/mL standard solution with acetone to 1 μmol/mL standard working solution.

D. Add reagents centrifuge tube in according to the following table

Reagent (μL)	Test Tube (A_T)	Standard Tube (A_S)	Control Tube (A_C)
Sample	all supernatant		
Standard working Solution		1000	
Solution I			1000
Solution II	100	100	100
Solution III	200	200	200
4000g，Centrifugal at RT for 10min, discard the supernatant and leave the precipitate			
Solution IV	1000	1000	1000

Add Solution 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, then pour it into the cuvette at 415nm, adjust the distilled water to zero, and the absorbance was recorded. The control tube need only be tested once or twice. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_C$.

3. Calculations

1. Bacteria, number of cells

   H_2O_2 content in bacteria or cells (μmol/10^4 bacteria or cells)

   $=\Delta A_T \div (\Delta A_S \div Cs) \times Vs \div (500 \times Vs \div Vst) = 0.002 \times \Delta A_T \div \Delta A_S$.

2. Fresh weight

   The content of H_2O_2 in tissue (with fresh weight of μmol/g)

   $=\Delta A_T \div (\Delta A_S \div Cs) \times Vs \div (Vs \div Vst \times W) = \Delta A_T \div \Delta A_S \div W$.

3. Protein concentration

   Content of H_2O_2 (μmol/mg prot)$=\Delta A_T \div (\Delta A_S \div Cs) \times Vs \div (Cpr \times Vs) = \Delta A_T \div \Delta A_S \div Cpr$.

4. Serum (plasma) volume

   The content of H_2O_2 in Serum (plasma)(μmol/mL)$= \Delta A_T \div (\Delta A_S \div Cs) \times Vs \div Vsp = 10 \times \Delta A_T \div \Delta A_S$.

500: number of cells in tens of thousands;

Cs: H_2O_2 standard solution concentration, 1μmol/mL;

Vs: sample volume added, 1mL;
W: fresh tissue, g;
Vst: volume used in the extraction process, 1mL;
Cpr: sample protein concentration, mg/mL;
V sp : volume of serum (plasma) used, 0.1ml.

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 0.9, it is recommended to dilute the sample with Reagent I before performing the measurement.

Experimental examples:

1. Take 0.1 g of shepherd's purse and add 1 mL of Reagent I for sample processing. After centrifugation to take all the supernatant, proceed according to the determination procedure. Calculate $\Delta A_T=A_T-A_C=0.109-0.003=0.106$, $\Delta A_S=A_S-A_C=0.637-0.003=0.634$. The content is calculated according to the sample mass.
   The content of H_2O_2 in tissue (with fresh weight of μmol/g) $=\Delta A_T\div\Delta A_S\div W=1.672$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. 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.
   The content of H_2O_2 in tissue (with fresh weight of μmol/g) $=\Delta A_T\div\Delta A_S\div W=4.022$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:

BC0020/BC0025	Malondialdehyde(MDA) Content Assay Kit
BC1090/BC1095	Xanthine Oxidase(XOD) Activity Assay Kit
BC0690/BC0695	Glucose Oxidase(GOD) Activity Assay Kit

Technical Specifications:

Minimum Detection Limit：0.002 μmol/mL

Linear Range：0.0097-1.5 μmol/mL