Hydrogen Peroxide(H2O2) Content Assay Kit

Product Code: 112862

Hydrogen Peroxide (H2O2) Content Assay Kit - Spectrophotometric Method

Product Information

Product code: 112862

Hydrogen peroxide (H2O2) is one of the most common reactive oxygen molecules in organisms. It is mainly produced by catalysts such as SOD and XOD and degraded by catalysts such as CAT and POD.

H2O2 is an important reactive oxygen species and a central molecule in the interconversion of reactive oxygen species. It can directly or indirectly oxidize intracellular nucleic acids, proteins, and other biological macromolecules, causing cell membrane damage and accelerating cellular aging and disintegration. H2O2 is also a key regulatory factor in many oxidative stress responses.

In this assay, H2O2 reacts with titanium sulfate to form a yellow peroxotitanium complex with characteristic absorbance at 415 nm.

Package List

Item	Name	Quantity	Storage
112862.1	Reagent I	0.3 g	2-8°C, protected from light
112862.2	Reagent II	12 mL	2-8°C
112862.3	Reagent III	60 mL	2-8°C
112862.4	Standard	1 mL	2-8°C, protected from light
112862.m	Instructions	1 copy	/

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Standard	--	--

Transportation and Storage

Transportation: This product is transported with ice packs.

Storage: Store according to the instructions. Shelf life is 180 days.

Materials Required but Not Provided

- Acetone, 60 mL, used as the extraction solution
- Concentrated hydrochloric acid, 6 mL
- Visible spectrophotometer
- Benchtop centrifuge
- Adjustable pipette
- 1 mL glass cuvette
- Mortar, homogenizer, or cell ultrasonic disruptor
- Ice

Sample Preparation

Bacteria or Cells

1. Collect bacteria or cells into a centrifuge tube, centrifuge, and discard the supernatant.
2. Add 1 mL acetone for every 5,000,000 bacteria or cells.
3. Disrupt the bacteria or cells by ultrasound at 20% power, 3 seconds ultrasound with 10 seconds interval, repeated 30 times.
4. Centrifuge at 8000g and 4°C for 10 min.
5. Collect the supernatant and keep it on ice for testing.

Tissue

1. Weigh approximately 0.1 g tissue.
2. Add 1 mL acetone and homogenize in an ice bath.
3. Centrifuge at 8000g and 4°C for 10 min.
4. Carefully collect all supernatant and keep it on ice for testing.

Serum or Plasma

1. Add 100 μL serum or plasma to 0.9 mL acetone and mix thoroughly.
2. Centrifuge at 8000g and 4°C for 10 min.
3. Carefully collect all supernatant and keep it on ice for testing.

> Because acetone is volatile, pre-cool it before use and perform grinding on ice. If evaporation is rapid, adjust the volume back to 1 mL with acetone after homogenization.

Reagent Preparation

Before use, add 6 mL concentrated hydrochloric acid to Reagent I and dissolve thoroughly. Store unused prepared reagent at 4°C.

Assay Procedure

1. Preheat the spectrophotometer for 30 min, set the wavelength to 415 nm, and zero with distilled water.
2. Place Reagent I, Reagent II, and Reagent III in a 37°C water bath for mammals or a 25°C water bath for other species for 10 min.
3. Dilute the standard before measurement as described below.

Standard Dilution

The standard is a 1 mmol/mL H2O2 solution. Dilute it with acetone to prepare 5, 2.5, 1.25, 0.625, 0.3125, 0.078, and 0.0195 μmol/mL standard solutions.

No.	Concentration Before Dilution (μmol/mL)	Standard Solution Volume (μL)	Acetone Volume (μL)	Concentration After Dilution (μmol/mL)
1	1000	10	1990	5
2	5	1000	1000	2.5
3	2.5	1000	1000	1.25
4	1.25	1000	1000	0.625
5	0.625	1000	1000	0.3125
6	0.3125	325	975	0.078
7	0.078	250	750	0.0195

> Each standard tube in the following experiment requires 1000 μL standard solution. Do not measure absorbance directly at this step.

Reaction Setup

Component	Blank Tube	Test Tube	Standard Tube
Acetone (μL)	1000	-	-

Component	Blank Tube	Test Tube	Standard Tube
Sample (μL)	-	All supernatant	-
Standard solution (μL)	-	-	1000
Reagent I (μL)	100	100	100
Reagent II (μL)	200	200	200

1. After adding the components listed above, centrifuge at 4000g at room temperature for 10 min.
2. Discard the supernatant and retain the precipitate. Acetone may be used to wash 3-5 times first to remove plant pigments.
3. Add 1000 μL Reagent III to each tube to dissolve the precipitate.
4. Let stand at room temperature for 5 min.
5. Transfer to a cuvette and measure absorbance at 415 nm. Record values as Ablank, Ameasured, and Astandard.

Calculate ΔA = Ameasured - Ablank and ΔAstandard = Astandard - Ablank. The blank tube and standard curve only need to be run 1-2 times.

Calculation of H2O2 Content

Standard Curve

Prepare the standard curve using the concentration of the standard tube as X, in μmol/mL, and absorbance as Y, using ΔAstandard. Substitute the sample ΔA value into the standard curve to calculate the sample concentration X, in μmol/mL.

Calculated by Number of Bacteria or Cells

H2O2 content (μmol/10^4 cells) = X × Vsample ÷ (N × Vsample ÷ Vextract) × F = X ÷ N × F

Calculated by Tissue Mass

H2O2 content (μmol/g mass) = X × Vsample ÷ (W × Vsample ÷ Vextract) × F = X ÷ W × F

Calculated by Protein Concentration

H2O2 content (μmol/mg prot) = X × Vsample ÷ (Vsample × Cpr) × F = X ÷ Cpr × F

Calculated for Serum or Plasma

H2O2 content (μmol/mL) = X × Vsample ÷ Vserum/plasma × F = 10 × X × F

Formula Definitions

- N: number of cells or bacteria, counted in ten thousands
- Vsample: sample volume added to the reaction system, 1 mL
- Vextract: volume of extraction solution added, 1 mL
- W: tissue mass, g
- Cpr: sample protein concentration, mg/mL
- Vserum/plasma: volume of serum or plasma used, 0.1 mL
- F: sample dilution factor

Precautions

1. This 50T kit can measure 48 samples. Before formal measurement, select 2-3 samples with large expected differences for a pre-test.
2. The linear range of this kit is 0.0195-5 μmol/mL.
3. The reagents in this kit are highly volatile. Wear disposable gloves and a mask.
4. Acetone denatures proteins. If calculation is based on protein concentration, extract tissue separately and determine the protein concentration again.

Appendix

For better accuracy, prepare a standard curve following the operating procedure above. The standard curve should have $R2 \geq 0.99$. Use the absorbance values of the 112862 standard tubes to prepare the curve and obtain the calculation formula for sample analysis.

Visual Reference