Hydrogen Peroxide (H2O2) Content Assay Kit - Microplate Method

Product Introduction

Hydrogen peroxide (H2O2) is one of the most common reactive oxygen species in organisms. It is mainly produced through catalytic reactions involving SOD, XOD, and related enzymes, and degraded by CAT, POD, and related enzymes.

H2O2 is both an important reactive oxygen species and a central molecule in the interconversion of reactive oxygen species. It can directly or indirectly oxidize intracellular biomacromolecules such as nucleic acids and proteins, damage cell membranes, and accelerate cellular aging and disintegration. It is also a key regulatory factor in many oxidative stress responses.

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

Package Contents

Product Code	Component	Quantity	Storage
112864.1	Reagent I	0.15 g	2-8 °C, protected from light
112864.2	Reagent II	6 mL	2-8 °C
112864.3	Reagent III	30 mL	2-8 °C
112864.4	Standard	1 mL	2-8 °C, protected from light
-	Instruction Manual	1 copy	-

Quality and Safety Information

Component	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 in this manual. Shelf life: 180 days.

Materials Required but Not Provided

- Acetone, 110 mL, used as extraction solution
- Concentrated hydrochloric acid, 3 mL
- Microplate reader
- Benchtop centrifuge
- Adjustable pipettes
- 96-well plate
- Mortar, homogenizer, or ultrasonic cell disruptor
- Ice

Assay Procedure

1. H_2O_2 Extraction

Bacteria or Cells

1. Collect bacteria or cells into a centrifuge tube, centrifuge, and discard the supernatant.
2. Add 500 x 10,000 bacteria or cells to 1 mL acetone.
3. Disrupt the bacteria or cells by ultrasound at 20% power, 3 seconds ultrasound, 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. Collect all supernatant carefully and keep it on ice for testing.

Serum or Plasma

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

Because acetone is volatile, pre-cool it before use. Grinding must be performed on ice. If acetone evaporates rapidly, bring the volume back to 1 mL with acetone after homogenization.

2. Reagent Preparation

Before use, add 3 mL concentrated hydrochloric acid to Reagent I and dissolve thoroughly. Store unused reagent at 4 °C.

3. Instrument and Reagent Preparation

1. Preheat the microplate reader for 30 min.
2. Set the wavelength to 415 nm and zero with distilled water.
3. Incubate Reagent I, Reagent II, and Reagent III in a water bath for 10 min at 37 °C for mammalian samples or 25 °C for other species.

4. Standard Dilution

The standard is a 1 mmol/mL H_2O_2 solution. Dilute the standard 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 requires 250 µL standard solution in the following experiment. Do not measure absorbance directly at this dilution step.

5. Measurement Procedure

Add the components to tubes according to the table below.

Component	Blank Tube	Measurement Tube	Standard Tube
Acetone (μL)	250	-	-
Sample (μL)	-	250	-
Standard solution (μL)	-	-	250
Reagent I (μL)	25	25	25
Reagent II (μL)	50	50	50

1. Centrifuge at 4000g at room temperature for 10 min.
2. Discard the supernatant and keep the precipitate. Acetone may be used to wash 3-5 times first to remove plant pigments.
3. Add 250 μL Reagent III to each tube to dissolve the precipitate.
4. Let stand at room temperature for 5 min.
5. Add the solution to a 96-well plate and measure absorbance at 415 nm.
6. Record the absorbance values as Ablank, Aassay, and Astandard.

Calculate ΔA = Aassay - Ablank and ΔAstandard = Astandard - Ablank.

The blank tube and standard curve only need to be run 1-2 times.

Calculation of H_2O_2 Content

1. Standard Curve

Use the standard tube concentration (X, μmol/mL) and absorbance (Y, ΔAstandard) to establish the standard curve. Substitute the sample ΔA value (Y, ΔA) into the standard curve formula to calculate the sample concentration (X, μmol/mL).

2. Calculation by Number of Bacteria or Cells

H_2O_2 content (μmol/10^4cells) = X x Vsample ÷ (N x Vsample ÷ Vextraction) x F = X ÷ N x F

3. Calculation by Tissue Mass

H_2O_2 content (μmol/g mass) = X x Vsample ÷ (W x Vsample ÷ Vextraction) x F = X ÷ W x F

4. Calculation by Protein Concentration

H_2O_2 content (μmol/mg prot) = X x Vsample ÷ (Vsample x Cpr) x F = X ÷ Cpr x F

5. Calculation for Serum or Plasma

H_2O_2 content (μmol/mL) = X x 10 x F

Formula Definitions

- N: number of cells or bacteria, in ten thousands
- Vsample: sample volume added to the reaction system, 0.25 mL
- Vextract: volume of extraction solution added, 1 mL
- W: tissue mass, g
- Cpr: sample protein concentration, mg/mL
- 10: serum dilution factor, [0.1 mL serum or plasma + 0.9 mL acetone] ÷ 0.1 mL serum or plasma = 10
- F: sample dilution factor

Precautions

1. This 100T kit can measure 96 samples. Before formal measurement, select 2-3 samples with expected large differences for a preliminary experiment.
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 during operation.

4. Acetone denatures proteins. If calculation is based on protein concentration, extract a separate tissue sample to determine the protein concentration.

Appendix

For greater accuracy, prepare a standard curve according to the operating steps above. The standard curve formula may be used, or the absorbance values from each standard well may be plotted to generate a standard curve with $R^2 \geq 0.99$ for sample calculation.

Visual Reference