Hydrogen Peroxide(H2O2) Content Assay Kit

Product Code: 112863

Hydrogen Peroxide (H_2O_2) Content Assay Kit, Micro Method

Product Information

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Hydrogen peroxide (H_2O_2) is a common reactive oxygen species in organisms. It is mainly produced by catalysts such as SOD and XOD and degraded by catalysts such as CAT and POD. $H_2O_2$is an important reactive oxygen species and a central molecule in the interconversion of reactive oxygen species.

H_2O_2can directly or indirectly oxidize intracellular nucleic acids, proteins, and other biomacromolecules, causing cell membrane damage and accelerating cellular aging and disintegration. It is also a key regulator in many oxidative stress responses.

H_2O_2reacts with titanium sulfate to form a yellow titanium peroxide complex with characteristic absorbance at 415 nm.

Example Performance

Sample measured: paper mulberry leaves subjected to low-temperature stress.

Measurement	$OD_{415\ nm}$
Blank	0.059
Sample	0.847 / 0.826

Actual readings may vary depending on the detection instrument and conditions. The values above are for reference only.

Package Contents and Storage

Pack size: 100T

Code	Component	Quantity	Storage
112863.1	Reagent I	0.15 g	2-8°C, protected from light
112863.2	Reagent II	6 mL	2-8°C
112863.3	Reagent III	30 mL	2-8°C
112863.4	Standard	1 mL	2-8°C, protected from light
Instruction manual	1 copy		

Quality and Safety Information

Material	Quality Standard	Main Toxicity
Reagent I	Not specified	Not specified
Reagent II	Not specified	Not specified
Reagent III	Not specified	Not specified
Standard	Not specified	Not specified

Transportation and Storage

Transportation: Transport with ice packs.

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

Materials Required but Not Supplied

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

Sample Preparation

Bacteria or Cells

1. Collect bacteria or cells into a centrifuge tube.
2. Centrifuge and discard the supernatant.
3. Add 1 mL acetone for every 5,000,000 bacteria or cells.
4. Disrupt by ultrasonication at 20% power: sonicate for 3 seconds, pause for 10 seconds, and repeat 30 times.
5. Centrifuge at 8000g and 4°C for 10 minutes.
6. Collect the supernatant and keep 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 minutes.
4. Collect all supernatant carefully and keep on ice for testing.

Serum or Plasma

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

> Acetone is volatile. Pre-cool acetone before use and perform grinding on ice. If evaporation is rapid, bring the volume back to 1 mL with acetone after homogenization.

Reagent Preparation

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

Assay Procedure

Instrument and Reagent Setup

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

Standard Dilution

The standard is a 1 mmol/mL H_2O_2solution. 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 in the following assay requires 250 μL standard solution. Do not measure absorbance directly at this dilution step.

Measurement

Add the following components to tubes as shown:

Component	Blank Tube	Test 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 minutes.
2. Discard the supernatant and retain 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 minutes.
5. Transfer to a micro glass cuvette or 96-well plate.
6. Measure absorbance at 415 nm and record the values as A_{blank}, A_{assay}, and $A_{standard}$.

Calculate $\Delta A = A_{assay} - A_{blank}$ and $\Delta A_{standard} = A_{standard} - A_{blank}$.

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

Calculation

Standard Curve

Use the standard tube concentration, X in μmol/mL, and absorbance, Y as $\Delta A_{standard}$, to establish the standard curve. Substitute the sample ΔA value into the standard curve to calculate the sample concentration, X in μmol/mL.

Calculation by Number of Bacteria or Cells

H_2O_2 content (μmol/10^4 cells) = $X \times V_{sample} \div (N \times V_{sample} \div V_{extraction}) \times F = X \div N \times F$

Calculation by Tissue Mass

H_2O_2 content (μmol/g mass) = $X \times V_{sample} \div (W \times V_{sample} \div V_{extraction}) \times F = X \div W \times F$

Calculation by Protein Concentration

H_2O_2 content (μmol/mg prot) = $X \times V_{sample} \div (V_{sample} \times Cpr) \times F = X \div Cpr \times F$

Calculation for Serum or Plasma

H_2O_2 content (μmol/mL) = $X \times 10 \times F$

Formula Definitions

- N: number of cells or bacteria, in ten-thousands
- V_{sample}: sample volume added to the reaction system, 0.25 mL
- $V_{extract}$: volume of extraction solution added, 1 mL
- W: tissue mass, g
- Cpr: sample protein concentration, mg/mL
- 10: serum dilution factor, calculated as [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 test 96 samples. Before formal measurement, select 2-3 samples expected to have 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.
4. Acetone will denature proteins. If calculation is based on protein concentration, prepare another tissue extract to re-determine the protein concentration.

Appendix

The standard curve is more accurate when prepared by the user. Use the operation table above to prepare the standard curve. The standard curve formula may be used, or the absorbance values from each standard well may be used to generate a standard curve with $R^2 \geq 0.99$ for sample calculations.

Visual Reference