Ver.241201

Hydrogen Peroxide (H_2O_2) Assay Kit

BC2201-01 (50 Tests)

FOR RESEARCH US ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

The HRP-TMB assay is a widely used colorimetric method for detecting and quantifying hydrogen peroxide (H_2O_2). It leverages the enzymatic activity of horseradish peroxidase (HRP) to catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H_2O_2 producing a blue-green coloured product. Acidic condition stops the reaction and changes the colour to yellow with maximum absorption at 450nm. The intensity of the colour produced is directly proportional to the amount of H_2O_2 in the sample.

Kit components

Components	Volume	Storage
Extraction Solution	60mL	4°C
Reagent I	120µL × 1	-20°C
Reagent II	12mL × 1	4°C
Reagent III	10mL × 1	4°C
Reagent IV	6mL × 1	4°C
Standard (H_2O_2: 1mM)	200µL × 1	4°C

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer / microplate reader, ultra-micro cuvette/96 well plate and distilled water.

Protocol

I. Sample Preparation

Tissue: Prepare 10% tissue homogenate by adding 1mL Extraction Solution to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 8000rpm, 4°C for 10 minutes and collect the supernatant.

Bacteria or cells: Harvest the cells and wash twice with Extraction Solution. Add 1mL ice cold Extraction Solution to 5 million cells and ultrasonicate (200W, work time 3s/ interval 10s repeat for 30 times) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 8000rpm, 4°C for 10 minutes and collect the supernatant. The supernatant should be kept on ice.

Serum or Plasma

Directly use for the assay

II. Assay procedure

- Preheat the spectrophotometer reader/microplate reader for 30 min, adjust wavelength to 450nm and set zero with distilled water.
- Reagent I Working Solution: Dilute Reagent I, 100-fold with Reagent II. Prepare just enough for the assay. For example, 10µL Reagent I with 990µL of Reagent II.

  Standard Preparation
- Prepare a 1:10 dilution of Hydrogen Peroxide Standard with Extraction Solution (mix 50µL of Standard with 450µL of Extraction Solution), and label as the Standard No.6 (100 µM).
- Make series of lower dilutions as described in the table.

No.	Concentration	Material needed
Standard 6	100µM	50µL of Standard + 450µL of Extraction Solution
Standard 5	50µM	200µL of Standard 6 + 200µL of Extraction Solution
Standard 4	25µM	200µL of Standard 5 + 200µL of Extraction Solution
Standard 3	12.5µM	200µL of Standard 4 + 200µL of Extraction Solution
Standard 2	6.25µM	200µL of Standard 3 + 200µL of Extraction Solution
Standard 1	3.125µM	200µL of Standard 2 + 200µL of Extraction Solution
Standard 0	0µM	200µL of Extraction

Note: Standard No.0 serves as the blank, B

- Pipette 50µL of either samples or standard.
- Add 100µL Reagent I Working Solution.
- Add 90µL of Reagent III
- Mix thoroughly, incubate at room temperature for 15 minutes.
- Add 50µL Reagent IV and immediately measure the absorbance of wavelength at 450nm. Record A_T, A_B and A_S.

  A_T: Absorbance of Test

  A_B: Absorbance of Blank

  A_S: Absorbance of Standard
- Subtract OD of Blank from OD of Standards and Tests

  $\Delta A_T = A_T - A_B$

  $\Delta A_S = A_S - A_B$

Calculations

- Create a standard curve with H_2O_2 concentration in the x-axis and OD @ 450nm in the y-axis. Plot the standard curve and get the slope of the curve from the equation y = kx + b. Input the ΔA_T into the equation to get x (µmol/L)
- Conversion Factor: 100µM of Hydrogen Peroxide is equivalent to 3.4 µg/mL.
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.