Hydrogen Peroxide (H_2O_2) Assay Kit

(BC043 Size:50T/48S)

1. Principle （Spectrophotometric Method）

Hydrogen peroxide (H_2O_2) can react with molybdate to form a complex. The amount of this complex can be measured at 405nm, from which the amount of H_2O_2 can be calculated.

2. Compositions and Preparation (The kit is valid fr 6 months)

Reagent I: 1 Bottle×60ml. Stored at 4℃and is stable for 6 months.

Reagent II: 1 Bottle×60ml. Preserved at 4 ℃ for 6 months. As a supersaturated solution, crystallization may occur and under such circumstance, dissolve the crystal at 60℃.

H_2O_2 standard solution: Stock solution 5 mL × 1 bottle, stored at 4℃; Before use, prepare the 163 mmol/L H_2O_2 standard solution by mixing the **H_2O_2 standard stock solution with distilled water in a ratio of 1:9**, and prepare it as needed.

3. Required Instruments and Reagents

Visible light spectrophotometer and a 1 cm optical path cuvette (or an enzyme detector (405nm) and a 96-well plate), distilled water.

4. Operation method

1. Sample pretreatment:

Liquid samples such as serum (plasma): Use directly.

Cell culture medium: Centrifuge a portion at 8000 revolutions per minute for 5 minutes, then take the supernatant for testing.

Animal tissue sample: Accurately weigh the tissue, add 9 times its volume of normal saline at a ratio of weight (g): volume (mL) = 1:9, perform mechanical homogenization under an ice water bath, centrifuge at 12,000 revolutions per minute for 10 minutes, and take the supernatant (the supernatant needs to be determined for its protein concentration, and the protein determination kit is available in our company, BC016) for testing.

Plant tissue samples: Method 1: First, clean the plant tissues with PBS, then dry them with absorbent paper, and cut them into pieces and put them into a mortar. Grind them into powder using liquid nitrogen. Weigh the plant powder, and add 9 times the volume of PBS at a ratio of (weight (g): volume (mL)) = 1:9. Vortex shake (or use a grinding instrument to grind) for 1 minute at 12,000 revolutions per minute, then centrifuge for 10 minutes. Take the supernatant for testing. Method 2: After washing and drying the samples, directly weigh them. Add 9 times the volume of PBS at a ratio of (weight (g): volume (mL)) = 1:9. Perform mechanical homogenization under an ice water bath, at 12,000 revolutions per minute, and centrifuge for 10 minutes. Take the supernatant for testing. (Note: Generally, for plants with higher moisture content, method 2 is used; conversely, for plants with lower moisture content or dry samples, method 1 is recommended for processing.)

Cell sample: After collecting the cells, each sample (the number of cells should be as high as possible, preferably no less than 106, the more the better) is added with 0.3 mL of physiological saline (or PBS), and then ultrasonicated under an ice water bath (power 200-300W, run for 5 seconds, with a 15-second interval, repeat 3-5 times). Centrifuge at 12,000 revolutions per minute for 10 minutes, and take the supernatant (the supernatant needs to be measured for its protein concentration, and the protein determination kit is available in our company, BC016) for testing.

2.Operation Table

Compositions (ml)	Blank	Standard	Sample
Reagent I	1	1	1
DDW	0.1		
163 mmol/L H_2O_2 Standard Solution		0.1	
Sample			0.1
Reagent II	1	1	1

Mix well. At 405nm, with a 1 cm optical path, zero the reading with DDW. Measure the absorbance values A of each tube (or take 200 μL of the reaction solution from each tube and add it to the 96-well plate, and read at 405nm with the microplate reader).

5. Calculation Formula and Examples

1.Calculation formula

$$\mathrm{H_2O_2\ Content\ in\ Liquid\ Sample\ (mmol/L)} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times C_{Standard} \times N$$

$$\mathrm{H_2O_2\ Content\ in\ Tissues/Cells\ (mmol/gprot)} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times C_{Standard} \times Cpr$$

$$\mathrm{H_2O_2\ Content\ in\ Tissues\ (mmol/g\ fresh\ weight)} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times C_{Standard} \times \frac{W}{V_{Sample}}$$

$C_{standard}$: Concentration of standard solution: 163 mmol/L;

N: Dilution factor of sample before testing

Cpr: Organized protein concentration, gprot/L (prot refers to protein).

W: Sample mass, g;

Vsample: The volume of the lysis medium added during sample pretreatment (lysis), L.

2. Calculation example:

Example 1: Take 0.1 mL of the original bovine serum directly and conduct the measurement according to the operation table. The absorbance of the measuring tube is 0.099, the absorbance of the blank tube is 0.006, and the absorbance of the standard tube is 0.341. Then the calculation result is:

$$\mathrm{H_2O_2\ Content\ in\ Bovine\ Serum\ (mmol/L)} = \frac{0.099 - 0.006}{0.341 - 0.006} \times 163 = 45.25\,\mathrm{mmol/L}$$

Example 2: Take 0.1 mL of 10% mouse lung homogenate and conduct the test according to the operation table. The absorbance of the test tube is measured to be 0.065, the blank tube's absorbance is 0.008, and the standard tube's absorbance is 0.346. At the same time, the protein concentration of 10% mouse lung homogenate is measured to be 6.284 gprot/L. Then the calculation result is:

$$\mathrm{H_2O_2\ content\ in\ Tissue\ (mmol/gprot)} = \frac{0.065 - 0.008}{0.346 - 0.008} \times 163 + 6.824 = 4.028\,\mathrm{mmol/gprot}$$

6. Notes

Some samples have their own inherent color or turbidity, so a control tube for the sample needs to be added on top of the operation table (i.e., 0.1 mL of the sample + 2 mL of PBS (or distilled water), mixed and read the value at 405 nm. When calculating, Ameasurement-Acomparison,and then substitute it into the calculation).

The sample volume should undergo a preliminary test. If the sample volume is too large and the absorbance exceeds 0.8, the sample must be diluted. If the sample volume is too small and the absorbance is less than 0.05, the sample volume needs to be increased. (The standard solution in the standard tube and the distilled water in the blank tube should both have the same amount increased, but the standard solution should be diluted accordingly before the increase is made.) The hydrogen peroxide level in the cells is relatively low. It is recommended that the number of cells be as large as possible (Minimum: not less than 1026).