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Hydrogen Peroxide(H2O2)Content Fluorometric Assay Kit

Hydrogen peroxide (H2O2) Content Fluorometric Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Fluorometric microplate reader

Catalog Number: AK1033

Size: 50T/48S ; 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time.

Reagent

50T size

100T size

Storage

Extract solution I

Liquid 60 mL×1

Liquid 110 mL×1

2-8℃

Extract solution II

Liquid 10 mL×1

Liquid 200 mL×1

2-8℃

Reagent I

Liquid 70 mL×1

Liquid 120 mL×1

2-8℃

Reagent II

Powder×1

Powder×2

-20℃

Reagent III

Liquid 0.08 mL×1

Liquid 0.12 mL×1

2-8℃

Standard

Liquid 0.5 mL×1

Liquid 0.5 mL×1

2-8℃

96-well black opaque plate

Plate×1

Plate×1

-

Sealing plate film (Cat#YA0249)

Film×2

Film×2

-

Solution Preparation:

1. Reagent II: Before use, one piece was added with 0.075 mL DMSO. After dissolution, the samples were divided according to the sample size. The samples used on the same day were stored at room temperature in the dark, and the rest were stored at-20 ℃ in the dark, which could be stored for 12 weeks. Please avoid repeated freezing and thawing. (The reagent is a freeze-dried reagent, there may be a large difference in the amount of reagents observed by the naked eye or even a small amount of the phenomenon, this phenomenon does not affect the use, the actual quality of the same.)

2. Working solution: Prepare according to the sample size in the ratio of Reagent I : Reagent II: Reagent III=480 μL : 10 μL : 10 μL(500 μL, 10T) before use. It needs to be stored away from light. The prepared working solution should be colorless and transparent. If the working solution is light pink, it is not available.

3. Standard: 400μmol/mL H2O2 standard solution. Before use, 0.01 mL of 400 μmol / mL H2O2 standard solution was taken, 0.99 mL of distilled water was added, and the mixture was fully mixed to obtain 4 μmol / mL H2O2 standard solution.

Note: The volume of reagent II, reagent III and standard is small, and the liquid is thrown off to the bottom by palm centrifuge before use.

Product Description:

Hydrogen peroxide(H2O2) is the most common reactive oxygen species in organisms, which is mainly catalyzed by SOD and XOD, and degraded by CAT and POD. H2O2 is not only one of the important reactive oxygen species, but also a hub for the mutual conversion of reactive oxygen species. On the one hand, H2O2 can directly or indirectly oxidize biological macromolecules such as nucleic acids and proteins in cells, damage cell membranes, and accelerate cell senescence and disintegration ; on the other hand, H2O2 is also a key regulator in many oxidative stress responses.

H2O2 reacts with the fluorescent probe under the action of the enzyme to produce a strong fluorescent product. The fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm is proportional to the H2O2 content.

Reagents and Equipment Required but Not Provided:

Fluorometric microplate reader, balance, desk centrifuge, pipette, mortar/homogenizer/cell ultrasonic crusher, 96-well black opaque plate(provided), sealing plate film(provided), EP tube, DMSO（AR≥98%）, ice and distilled water.

Operation procedure:

I. Sample preparation:

1. Tissue sample: ① Add Reagent I according to the ratio of tissue mass (g) : Reagent I volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL of Extract solution I), after ice bath homogenization, centrifuge at 4℃, 10000×g for 10min, take the supernatant (If the protein content is used to calculate the protein content of the sample in the later stage, the sample can be retained for determination at

this time). ②Take 0.1mL of supernatant and add 0.1mL of Extract solution I, centrifuge at 10000×g for 10 minutes at 4℃, take 0.8mL of supernatant and add 0.15mL Extract solution
II slowly until no bubbles. Then centrifuge at 10000g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

2. Bacteria/cells: ① Add Reagent I according to the number of bacteria/cells (106): the volume of Reagent I (mL) = 5-10:1 (it is recommended to add 1 mL of the Reagent I 5×106 million bacteria/cells), ultrasonic wave breaks bacteria/cells (power 200W, work time 3s, interval 6s, total time 3min). Then centrifuge at 4℃, 10000×g for 10min, take the supernatant (If the protein content is used to calculate the protein content of the sample in the later stage, the sample can be retained for determination at this time).

②Take 0.1mL of supernatant and add 0.1mL of Extract solution I, centrifuge at 12000×g for 10 minutes at 4℃, take 0.8mL of supernatant and add 0.15mL Extract solution II slowly until no bubbles. Then centrifuge at 10000×g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

3. Serum (plasma) or other liquid samples: ①If the protein content is used to calculate the protein content of the sample in the later stage, the sample can be retained for determination at this time.

②Take 0.1mL of liquid and add 0.1mL of Extract solution I, centrifuge at 12000×g for 10 minutes at 4℃, take 0.8mL of supernatant and add 0.15mL Extract solution II slowly until no bubbles. Then centrifuge at 10000×g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

II. Determination procedure:

1. Preheat the fluorometric microplate reader for more than 30min, adjust excitation wavelength/ emission wavelength to 535nm/587nm.

2. Equilibrate the working solution to room temperature before use.

3. Standard dilution: Dilute 4 μmol/mL(4000nmol/mL) standard solution with distilled water to obtain 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 nmol/mL standard solution before use.

4. Add reagents with the following list:

Reagent (μL)
Test tube
Standard tube
Blank tube
Sample
50
-
-
Standard
-
50
-
Distilled water
-
-

50
Working solution
50
50
50
Mix well and react in the dark at room temperature for 10 minutes. Measure Ex/Em=535nm/587 nm, recording as RFUT, RFUS and RFUB respectively. Calculate ΔRFUT = RFUT-RFUB, ΔRFUS = RFUS-RFUB. Blank tube and standard curve only need to be measured 1-2 times.

III. Calculations:

1.

Standard curve

According to the concentration of the standard tube (x, nmol/mL) and the absorbance ΔRFUS (y, ΔRFUS), a standard curve was established. According to the standard curve, ΔRFUT (y, ΔRFUT) was brought into the formula to calculate the sample concentration (x, nmol/mL).

2.

Calculation of H2O2 Content

1) Sample mass:

H2O2 content(nmol/g mass)= x×(Vsp+VII)÷[VL×Vsp÷(VI+VL)]×VRI÷W×F=13.06×x÷W×F

2) Bacteria/cells number:

H2O2 content(nmol/106 cell)= x×(Vsp+VII)÷[VL×Vsp÷(VI+VL)]×VRI÷N×F=13.06×x÷N×F

3) Protein concentration:

H2O2 content(nmol/g prot)= x×(Vsp+VII)÷[VL×Vsp÷(VI+VL)]×VRI÷(Cpr×VRI)×F=13.06×x÷Cpr×F

4) Liquid sample volume:

H2O2 content(nmol/mL)= x×(Vsp+VII)÷[VL×Vsp÷(VI+VL)]×F=13.06×x×F

Vsp: After adding the extract solution I, take the volume of the supernatant, 0.8 mL;

VII: Extract solution II volume, 0.15 mL;

VI: Extract solution I volume, 1 mL;

VL: Volume of liquid sample added to Extract solution I, 0.1 mL;

VRI: Volume of Reagent I added to the sample, 1mL;

W: Sample mass, g;

Cpr: Sample protein concentration, mg/mL(Protein concentration needs to be Self- determined);

N: Number of cells, count by 106;

F: Dilution ratio.

Notes:

1.

If the measured value is too small, the sample size can be increased or the sample size on the sample table can be increased during the pretreatment. If the measured value exceeds the linear range, the sample can be diluted with distilled water and then measured. The calculation formula needs to be modified synchronously.

2.

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Differences in the sensitivity of various fluorometric microplate readers may lead to variations in the detected RFU, which is a normal phenomenon. Provided that the reagent preparation and experimental procedure are correct and the standard curve exhibits good linearity, the detection results are considered accurate and valid.

Experimental examples:

1.

Dilute 400 μmol/L standard solution with distilled water to obtain 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 nmol/mL standard solution. Measure RFU of black tube and standard tubes, then establish the standard curve y = 13,375,676.60x+106,406.43, R2=0.9997.

2.

Results of different samples:

Samples

Mass/cell count

Dilution multiple

H2O2 Content

Unit

Rat liver

0.1g

1

112.24 nmol/g mass

Rat serum

-

1

2.34 nmol/mL

Jurkat

10×106 cell

1

0.189 nmol/mL