Superoxide Anion Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC1295

Size: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 Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 10 mL×1	2-8℃
Reagent II	Liquid 7 mL×1	2-8℃
Reagent III	Liquid 7 mL×1	2-8℃
Reagent IV	Requird but not provided	-
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. Reagent IV: self-prepared chloroform, about 12mL, stored at room temperature; An empty brown 30mL bottle is provided in the kit. Please label the reagent name by yourself.
2. Standard goods: 10μmol/mL sodium nitrite.
3. Preparation of 0.03125μmol/mL standard: Take 100μL 10μmol/mL sodium nitrite standard and add 900μL distilled water to dilute it into 1μmol/mL standard; Then take 30μL 1μmol/mL standard and dilute it with 930μL distilled water to 0.03125μmol/mL standard for standard tube determination in the following operating table.

Product Description:

Active oxygen such as superoxide anion in the living body has the functions of immunity and signal transduction. But if it accumulates too much, it will destroy the cell membrane and biomacromolecules, leading to abnormal metabolism of the cells and tissues of the body, and cause many diseases.

The superoxide anion reacts with hydroxylamine hydrochloride to form NO^{2-}, and the NO^{2-} under the action of p-aminobenzenesulfonamide and naphthalene ethylenediamine hydrochloride is produced a red azo compound with a characteristic absorption peak at 530 nm. The content of O^{2-} can be calculated according to the A530 value.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, water-bath, balance, mortar/homogenizer, centrifuge, micro glass cuvette/96 well flat-bottom plate, chloroform (>98%, AR) and distilled water.

Sample preparation:

1. Plant and animal tissues: Add Extract solution according to the ratio of tissue mass (g) : Extract

solution (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Extract solution), after ice bath homogenization, centrifuge at 4℃, 12000rpm for 10min, take supernatant and placed on the ice for test.

2. Bacteria or cell: The ratio of bacteria/cell amount (10^6): the volume of Extract solution (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s，interval 10 s，repeat 30 times). Centrifuge at 12000rpm for 10 minutes at 4℃, take the supernatant and placed on the ice for test
3. Serum or culture medium: detect directly. (If the solution is turbid, centrifuge to take the supernatant and then measure).

Procedure:

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 530 nm and set the counter to zero with distilled water.
2. Operation table:（Add the following reagents in turn to the 1.5mL EP tube）

Reagent name (μL)	Blank tube (A_B)	Test tube (A_T)	Standard tube (A_S)
Standard			100
Sample		100	-
Distilled water	100	-	-
Reagent I	80	80	80
Mix and react for 20 min at 37℃			
Reagent II	60	60	60
Reagent III	60	60	60
Mix and react for 20 min at 37℃			
Reagent IV	100	100	100
Mix well, centrifuge at 8000 rpm for 5 min at 25℃, carefully suck 200 μL of the upper water phase into micro glass cuvette/96 well flat-bottom plate, adjust zero with distilled water, measure the absorbance value at 530 nm, calculate the $\Delta A_S=A_S-A_B$, the $\Delta A_T=A_T-A_B$. Only one blank tube is needed for each experiment.			

Calculation:

1.Calculated according to the fresh weight of the sample

Superoxide anion content (μmol/g mass) = $2\times\Delta A_T\div(\Delta A_S\div C_S)\times Vst\div W$

$= 0.0625\times\Delta A_T\div\Delta A_S\div W$

Rate of superoxide anion production (μmol/min/g mass) =$2\times\Delta A_T\div(\Delta A_S\div C_S)\times Vst\div W\div T$

$= 0.003125\times\Delta A_T\div\Delta A_S\div W$

(2) Calculated by protein concentration

Superoxide anion content (μmol/mg prot) = $2\times\Delta A_T\div(\Delta A_S\div C_S)\times Vs\div(Vs\times Cpr)$

$=0.0625\times\Delta A_T\div\Delta A_S\div Cpr$

Rate of superoxide anion production (μmol/min/mg prot) = $2\times\Delta A_T\div(\Delta A_S\div C_S)\times Vs\div(Vs$

×Cpr) ÷ T

$= 0.003125 \times \Delta A_T \div \Delta A_S \div Cpr$

(3) Calculated according to the volume of serum or culture medium

Superoxide anion content (μmol/mL) = $2 \times \Delta A_T \div (\Delta A_S \div C_S) = 0.0625 \times \Delta A_T \div \Delta A_S$

Rate of superoxide anion production (μ mol/min/mL) = $2 \times \Delta A_T \div (\Delta A_S \div C_S) \div T = 0.003125 \times \Delta A_T \div \Delta A_S$

(4) Calculate by the number of bacteria or cells

Superoxide anion content (μmol/10^6 cell) = $2 \times \Delta A_T \div (\Delta A_S \div C_S) \times Vst \div N = 0.0625 \times \Delta A_T \div \Delta A_S \div N$ Rate of superoxide anion production (μmol/min/10^6 cell) = $2 \times \Delta A_T \div (\Delta A_S \div C_S) \times Vst \div N \div T$

$= 0.003125 \times \Delta A_T \div \Delta A_S \div N$

C_S: Standard tube concentration, 0.03125μmol/mL;

Vs: sample volume added, 0.04 mL;

Vst: volume used in the extraction process, 1 mL;

Cpr: sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

N: Number of cells/bacteria, measured in 10^6;

T: React time, 20 min.

2: Two molecules of O^{2-} react to produce one molecule of NO^{2-}.

Note:

1. If the ΔA <0.01, it is recommended to increase the sample size or extend the reaction time of the first step after the determination; If the ΔA >1.2, it is recommended to dilute the sample for determination, and pay attention to the simultaneous modification of the calculation formula
2. After the sample prepared, measure it immediately. Do not store the sample at low temperature for a long time to avoid affecting the measurement results.
3. Reagent IV has certain toxicity. Please take protective measures when operating. As the reagent has certain toxicity, please take protective measures during operation

Examples:

1. Add 0.1020g mouse liver to 1mL Extract solution and mix thoroughly, centrifuge with 12000rpm at 4℃ for 10min, take supernatant, follow the determination procedure to operate, and calculate: $\Delta A_T = A_T - A_B = 0.415 - 0.040 = 0.375$, $\Delta A_S = A_S - A_B = 0.362 - 0.040 = 0.322$, according with mass of sample to calculate superoxide anion content（μmol/g mass）== $0.0625 \times \Delta A_T \div \Delta A_S \div W = 0.714$ μmol/g mass.
2. Add 0.1045g Albizzia root to 1mL Extract solution and mix thoroughly, centrifuge with 12000rpm at 4℃ for 10min, take supernatant, follow the determination procedure to operate, and

calculate: $\Delta A_T = A_T - A_B = 0.116 - 0.040 = 0.076$, $\Delta A_S = A_S - A_B = 0.362 - 0.040 = 0.322$, according with mass of sample to calculate superoxide anion content（μmol/g mass）$= 0.0625 \times \Delta A_T \div \Delta A_S \div W = 0.141$ μmol/g mass.

3. Take 100μL mouse serum, according to the measurement procedure, use 96 well plate to measure the calculation $\Delta A_T = A_T - A_B = 0.415 - 0.040 = 0.375$, $\Delta A_S = A_S - A_B = 0.362 - 0.040 = 0.322$, calculate the superoxide anion content according to the liquid sample volume: superoxide anion content (μmol/mL) $= 0.0625 \times \Delta A_T \div \Delta A_S = 8.15 \times 10\text{-}3$ μmol/mL.

Recent Product citations：

[1] Bingbing Cai,Qiang Li,Fengjiao Liu,et al. Decreasing fructose-1,6-bisphosphate aldolase activity reduces plant growth and tolerance to chilling stress in tomato seedlings. physioogia plantarum. December 2017;

[2] Zhongyuan Liu,Peilong Wang,Tengqian Zhang,et al. Comprehensive analysis of BpHSP genes and their expression under heat stresses in Betula platyphylla. Environmental and Experimental Botany. August 2018;(IF3.712)

References：

[1] 王爱国，罗广华. 植物的超氧物自由基与羟胺反应的定量关系[J]. 植物生理学通讯, 1990, 6(3): 55-57.

Related products：

BC1090/BC1095	Xanthine Oxidase(XOD) Activity Assay Kit
BC0690/BC0695	Glucose Oxidase (GOD) Activity Assay Kit
BC1270/BC1275	Protein Carbonyl Content Assay Kit
BC1280/BC1285	Diamine Oxidase(DAO) Activity Assay Kit

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