Superoxide Anion Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC1290

Size:50T/48S

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 60 mL×1	2-8℃
Reagent I	Liquid 25 mL×1	2-8℃
Reagent II	Liquid 20 mL×1	2-8℃
Reagent III	Liquid 20 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 30mL, 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, water-bath, balance, mortar/homogenizer, centrifuge, 1 mL glass cuvette, chloroform and distilled water.

Sample preparation:

1. Plant and animal tissues: Weigh about 0.1 g of sample, add 1 mL of Extract solution and fully grind.

Centrifuge at 12000 rpm and 4°C for 20 min, then take 20 μL of supernatant to determine protein content, and the other supernatants as samples to be tested.

2. Serum or culture medium: detect directly.

Procedure:

1. Preheat spectrophotometer for 30min, adjust the wavelength to 530 nm and set the counter to zero with distilled water.
2. Prepared standard solution: Take a proper amount of sodium nitrite standard solution, first dilute it 8 times to 0.625 μmol/mL, then dilute it to 0.3125, 0.15625, 0.078, 0.039, 0.0195, 0.009765, 0.0049, 0.00244, 0.0012, 0.0006 μmol/mL gradient standard solution, and use 0.3125, 0.15625, 0.078, 0.039, 0.0195, 0.0097625, 0.00244, 0.0006 μmol/mL standard tube as standard curve.
3. Operation table:

Reagent name (mL)	Blank tube (Ab)	Test tube (At)	Standard tube (As)
Standard			0.2
Sample		0.2	
Extract solution	0.5	0.3	0.3
Reagent 1	0.4	0.4	0.4
Mix and react for 20 min at 37°C			
Reagent 2	0.3	0.3	0.3
Reagent 3	0.3	0.3	0.3
Mix and react for 20 min at 37°C			
Reagent 4	0.5	0.5	0.5
Mix well, centrifuge at 8000 rpm for 5 min at 25°C, carefully suck 1 mL of the upper water phase into 1 mL glass cuvette, adjust zero with distilled water, measure the absorbance value at 530 nm, calculate the $\Delta A_S=A_S-A_B$, the $\Delta A_S=A_T-A_B$. Only one blank tube is needed for each experiment.			

Calculation:

1. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis, ΔAs as Y-axis. Take ΔA_T into the equation to obtain x (mg/mL).
2. Calculation of superoxide anion content

   Take ΔA sample into the equation to get x value (μ mol/mL)

(1) Calculated according to the fresh weight of the sample

The content of superoxide anion (μmol/g fresh weight) = $2x\times V_S\div (V_S\div V_E\times W) = 2x \div W$.

The production rate of superoxide anion (μmol/min/g fresh weight)=$2x\times V_S\div (V_S\div V_E\times W)\div T=0.1x\div W$.

(2) Calculated by protein concentration

Superoxide anion content (μ mol/mg prot) =$2x\times V_S\div (V_S\times Cpr) =2x\div Cpr$.

The production rate of superoxide anion (μmol/min/mg prot) = 2x×V_S÷ (V_S× Cpr) ÷ T= 0.1x ÷ Cpr.

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

Superoxide anion content (μmol/mL) = 2x

The production rate of superoxide anion (μ mol/min/mL) =2x÷T = 0.1x.

Vs: sample volume added, 0.2 mL;

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

Cpr: sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

T: React time, 20 min.

Note:

1. Dilute sample with extract solution if OD>1.0. The sample shall be diluted properly and then determined. Pay attention to multiply the dilution times in 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.

Examples:

1. Add 0.1g mouse liver to 1mL extract solution and mix thoroughly, centrifuge with 12000rpm at 4℃ for 20min, take supernatant, follow the determination procedure to operate, and calculate: ΔA =A(T)-A(B)=0.252-0.008=0.244, standard curve: y=5.1285x+0.013 , calculate x=0.045, according with mass of sample to calculate superoxide anion content（μmol/g mass）=2x÷W=0.9 μmol/g mass.
2. Add 0.1g hibiscus to 1mL extract solution and mix thoroughly, centrifuge with 12000rpm at 4℃ for 20min, take supernatant, follow the determination procedure to operate, and calculate: ΔA =A(T)-A(B)=0.036-0.008=0.028, standard curve: y=5.1285x+0.013 , calculate x=0.0029, according with mass of sample to calculate superoxide anion content（μmol/g mass）=2x÷W=0.058 μmol/g mass.

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

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com