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:

Extract solution: 110 mL ×1, storage at 4℃.

Reagent 1: 12 mL×1, storage at 4℃.

Reagent 2: 8 mL×1, storage at 4℃.

Reagent 3: 8 mL×1, storage at 4℃.

Reagent 4: Chloroform, self-provided reagent.

$NaNO_2$ Standard: 0.5mL ×1, storage at 4℃. 10 μmol/mL $NaNO_2$ standard solution.

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 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 for 20 min at 4°C, 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/microplate reader for 30 min, 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 1.25 μmol/mL, then dilute it to 0.625, 0.3125, 0.15625, 0.078, 0.039, 0.0195, 0.009765, 0.0049, 0.00244, 0.0012 μmol/mL gradient standard solution, and use 0.625, 0.3125, 0.15625, 0.078, 0.039, 0.0195, 0.0049, 0.0012 μmol/mL standard tube as standard curve.

3. Operation table:

Reagent name (μL)	Blank tube (Ab)	Test tube (At)	Standard tube (As)
Standard			40
Sample		40	
Extract solution	100	60	60
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 $\Delta A_S=A_T-A_B$. 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) = $2x\times V_S\div (V_S\times Cpr) \div T= 0.1x \div Cpr$.

(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) =$2x\div T = 0.1x$.

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;

T: React time, 20 min.

Note:

1. Dilute sample with extract solution if OD>1.5. 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)=0.176-0.043=0.133, standard curve: y=2.9968x+0.0129 , calculate x=0.04, according with mass of sample to calculate superoxide anion content（μmol/g mass）=2x÷W=0.8 μmol/g mass.

2. Add 0.1g leaf 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)=0.091-0.043=0.048, standard curve: y=2.9968x+0.0129, calculate x=0.012, according with mass of sample to calculate superoxide anion content（μmol/g mass）=2x÷W=0.24 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