Superoxide Anion Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0770

Size:50T/48S

Product composition:

Extract solution: Liquid 100 mL×1, Storage at 4℃.

Reagent I: Liquid 32 mL×1, Storage at 4℃.

Reagent II: Liquid 25 mL×1, Storage at 4℃ and protected from light.

Reagent III: Liquid 25 mL×1, Storage at 4℃ and protected from light.

Reagent IV: Chloroform, self-provided reagent.

Standard: Liquid 1 mL×1, 10 μmol/mL $NaNO_2$, Storage at 4℃.

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℃			
Reagent 2	0.3	0.3	0.3
Reagent 3	0.3	0.3	0.3
Mix and react for 20 min at 37℃			
Reagent 4	0.5	0.5	0.5
Mix well, centrifuge at 8000 rpm for 5 min at 25℃, 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) = $2x\times V_S\div (V_S\times Cpr) \div T= 0.1x \div Cpr$.

(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, 0.2 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：

NA0789/NA0548	Xanthine Oxidase(XOD) Activity Assay Kit
NA0814/NA0572	Glucose Oxidase (GOD) Activity Assay Kit
NA0772/NA0531	Protein Carbonyl Content Assay Kit
NA0771/NA0530	Diamine Oxidase(DAO) Activity Assay Kit