CheKine™ Superoxide Anion Colorimetric Assay Kit

Cat #: KTB1210 Size: 48 T/96 T

	Superoxide Anion Colorimetric Assay Kit		
REF	**Cat #:** KTB1210	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant		
	Storage: Stored at 4℃ for 6 months and protected from light		

Assay Principle

Reactive oxygen species such as superoxide anions in organisms have immune and signal transduction effects, but when they accumulate too much, they will destroy cell membranes and biological macromolecules, leading to abnormal metabolism of cells and tissues in the body, causing many diseases. The superoxide anion in plant, animal tissues, serum and other samples reacts with hydroxylamine hydrochloride to produce NO_2^-, and NO_2^- reacts with Gris' reagent. The mechanism of Gris analysis is summarized as the azo coupling between diazoniums, which is It is produced by sulfonamides and NO_2^- and N-(1-naphthyl) ethylenediamine dihydrochloride to generate a red azo compound with a characteristic absorption peak at 540 nm. The O_2^- content in the sample can be calculated based on the A540 value. The kit can detect samples of plants, animal tissues, serum and cells.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	4 mL	8 mL	4°C
Reagent Ⅱ	3 mL	6 mL	4°C, Protected from light
Reagent Ⅲ	3 mL	6 mL	4°C, Protected from light
$NaNO_2$ Standard (10 mmoL/L)	0.5 mL	0.5 mL	4°C

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 540 nm
- Refrigerated Centrifuge, Ice Maker, Precision Pipettes, Disposable Pipette Tips
- Trichloromethane
- Deionized water

- 96-well plate with clear flat bottom
- Dounce homogenizer (for Tissue Samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

ReagentⅢ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Setting of standard curves: Dilute 20 μL of $NaNO_2$ Standard (10 mmoL/L) to 200 μmol/L with 980 μL Extraction Buffer. And dilute the standard furtherly with Extraction Buffer as shown in the table below:

	Volume of 200 μmol/L $NaNO_2$ Standard	Volume of Extraction Buffer	Concentration
Std.1	200 μL	0	200 μmoL/L
Std.2	100 μL	100 μL	100 μmoL/L
Std.3	50 μL	150 μL	50 μmoL/L
Std.4	20 μL	180 μL	20 μmoL/L
Std.5	10 μL	190 μL	10 μmoL/L
Std.6	5 μL	195 μL	5 μmoL/L
Std.7	2 μL	198 μL	2 μmoL/L
Std.8	1 μL	199 μL	1 μmoL/L

Sample Preparation

1. Animal Tissues: According to the ratio of tissue weight (g): The Extraction Buffer volume (mL) at 1: 5~10, it is recommended to weigh about 0.1 g tissue and add 1mL Extraction Buffer. Homogenize on ice. Centrifuge at 12,000rpm for 10 min at 4℃, aspirating the supernatant, place it on ice to be tested.

2. Plant Tissues: According to the ratio of plant weight (g): The Extraction Buffer volume (mL) at 1: 5~10, it is recommended to weigh about 0.1 g plant and add 1 mL Extraction Buffer. Homogenize or Ultrasonically break on ice (power 300w, ultrasound 3s, intermittent 7 s, total time 3 min). Centrifuge at 12,000 rpm for 10 min at 4℃, aspirating the supernatant, place it on ice to be tested.

3. Cells: Collect cells into a centrifuge tube (Suspension cells: Centrifuge at 800 g for 3 min and discard supernatant; Adherent cells: Place dish on ice, harvest using proteolytic enzymes or a rubber policeman), Wash cells with cold PBS, then centrifuge at 800 g for 3 min and discard the supernatant after centrifugation. According to the ratio of the cells number (10^4): the Extraction Buffer volume (mL) at 500~1,000: 1, it is recommended to add 1 mL Extraction Buffer for every 5×10^6 bacteria or cells. Homogenize or Ultrasonically break on ice (power 300 W, work 3 s, intermittent 7 s, total time 3 min). Centrifuge at 12,000 rpm for 10 min at 4℃, aspirating the supernatant, place it on ice to be tested.

4. Serum, Plasma, Cell Supernatant or other Liquid samples: Tested directly by adding samples to the microplate.

Assay Procedure

1．Preheat the microplate reader for more than 30 min, and adjust the wavelength to 540 nm.

2．Operation Table:

	Control Tube（μL）	**Blank Tube（μL）**	**Test Tube（μL）**	**Standard Tube（μL）**
Different concentration of Std.	0	0	0	40

Sample	40	0	40	0
Extraction Buffer	140	100	60	60
Reagent Ⅰ	0	80	80	80
Mix well, incubate in 37℃ water bath for 20 min				
Reagent Ⅱ	60	60	60	60
Reagent Ⅲ	60	60	60	60
Mix well, incubate in 37℃ water bath for 20 min				
Trichloromethane	100	100	100	100
Mix well, Centrifuge at 8,000 rpm for 5 min at 25℃, Add 200 μL into a 96-well plate, measure the absorbance value at 540 nm with a microplate reader and record it as A. $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: Each sample needs to set up a control well to eliminate the influence of NO_2^- existing in the sample itself, so 96 T can only measure 48 samples. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1．Drawing of standard curve:

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve y=kx+b.

2．Calculation of Superoxide Anion content:

Bring the ΔA_{Test} of the sample into the equation to get the y value (μmol/L). If the ΔA_{Test} of the sample is greater than 1.0, the sample needs to be appropriately diluted with the extraction buffer before measurement, and the calculated y value is multiplied by the dilution factor.

(1) Calculated by protein concentration

Superoxide Anion content(μmol/mg prot)=$y\times V_{Sample}\div(V_{Sample}\times Cpr)\times 10^3$=**1000y÷Cpr**

Superoxide Anion production rate(μmol/ min/mg prot) =$y\times V_{Sample}\div(V_{Sample}\times Cpr)\div T\times 10^3$=**50y÷Cpr**

(2) Calculated by fresh weight of samples

Superoxide Anion content(μmol/g fresh weight)=$y\times V_{Sample}\div(V_{Sample}\div V_{Sample\ Total}\times W)\times 10^3$=**1000y÷W**

Superoxide Anion production rate(μmol/min/g fresh weight)=$y\times V_{Sample}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T\times 10^3$=**50y÷W**

(3) Calculated by volumet of liquid samples:

Superoxide Anion content(μmol/mL)=$y\times 10^3$=**1000y**

Superoxide Anion production rate(μmol/min/mL)=$y\div T\times 10^3$=**50y**

Where: V_{Sample}: sample volume added, 0.04 mL; Cpr: sample protein concentration, mg/mL; T: reaction time, 20 min; $V_{Sample\ Total}$: extract buffer added to samples, 1 mL; W: sample weight, g; 10^3: 1 L=10^3 mL.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

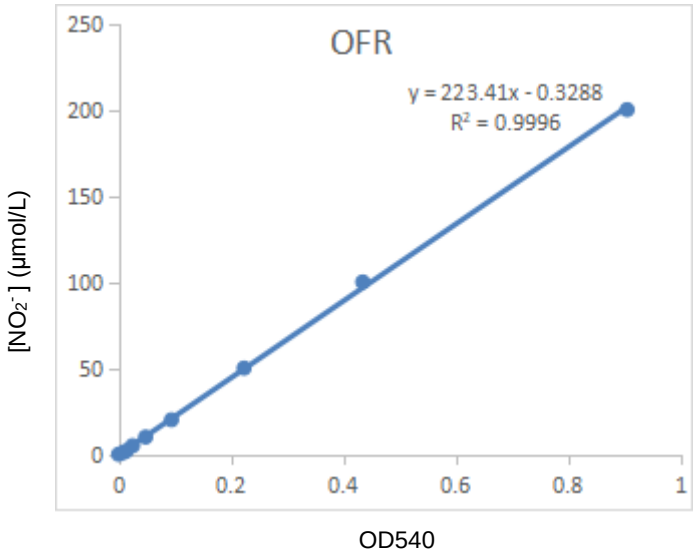

Fig. Standard Curve of Superoxide Anion assay.

Recommended Products

Catalog No.	Product Name
KTB1050	CheKine™ Lipid Peroxidation (MDA) Assay Kit
KTB1041	CheKine™ Hydrogen Peroxide (H_2O_2) Assay Kit
KTB1310	CheKine™ Glucose Oxidase Activity (GOD) Assay Kit
KTB1070	CheKine™ Xanthine Oxidase Assay Kit
KTB1200	CheKine™ Protein Carbonyl Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.