



## CheKine™ Micro Superoxide Anion Assay Kit

Cat #: KTB1210

Size: 48 T/96 T

	<b>Micro Superoxide Anion Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB1210	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable samples:</b> Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant		
	<b>Storage:</b> Stored at 4°C for 6 months, 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  $\text{NO}_2^-$ , and  $\text{NO}_2^-$  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  $\text{NO}_2^-$  and N-(1-naphthyl) ethylenediamine dihydrochloride to generate a red azo compound with a characteristic absorption peak at 540 nm. The  $\text{O}_2^-$  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°C
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
$\text{NaNO}_2$ Standard (10 mmol/L)	0.5 mL	0.5 mL	4°C

### Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, ice maker, water bath
- Trichloromethane, deionized water

- Homogenizer (for tissue samples)

## Reagent Preparation

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

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

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

**Reagent III:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

**Setting of standard curves:** Dilute 20 µL of NaNO<sub>2</sub> 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:

Num.	Volume of 200 µmol/L NaNO <sub>2</sub> 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: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
2. Plant Tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
3. Cells: Collect 5×10<sup>6</sup> cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
4. Serum, Plasma, Cell Supernatant or other liquid samples: Tested directly.

**Note:** It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, visible spectrophotometer was returned to zero with deionized water.
2. Operation table:

Reagent	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
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Mix well, incubate in 37°C water bath for 20 min

Reagent II	60	60	60	60
Reagent III	60	60	60	60

Mix well, incubate in 37°C water bath for 20 min

Trichloromethane	100	100	100	100
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Mix well, Centrifuge at 8,000 g for 5 min at 25°C, Add 200 µL into 96-well plate or microglass cuvette, measure the absorbance value at 540 nm and record it as A.  $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}}$ ,  $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$ .

**Note: Each sample needs to set up a control tube to eliminate the influence of NO<sub>2</sub><sup>-</sup> 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. If  $\Delta A_{\text{Test}}$  is less than 0.005, increase the sample quantity appropriately. If  $\Delta A_{\text{Test}}$  is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.**

## 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  $\Delta A_{\text{Standard}}$  as the x-axis, draw the standard curve  $y=kx+b$ . Bring the  $\Delta A_{\text{Test}}$  of the sample into the equation to get the y value (µmol/L).

### 2. Calculation of Superoxide Anion content

#### (1) Calculated by protein concentration

Superoxide Anion content (µmol/mg prot) =  $y \times V_{\text{Sample}} \div (V_{\text{Sample}} \times \text{Cpr}) \times 10^{-3} = \mathbf{y \div \text{Cpr}}$

Superoxide Anion production rate (µmol/ min/mg prot) =  $y \times V_{\text{Sample}} \div (V_{\text{Sample}} \times \text{Cpr}) \div T \times 10^{-3} = \mathbf{0.05y \div \text{Cpr}}$

#### (2) Calculated by fresh weight of samples

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

Superoxide Anion production rate (µmol/min/g fresh weight) =  $y \times V_{\text{Sample}} \div (V_{\text{Sample}} \div V_{\text{Sample Total}} \times W) \div T \times 10^{-3} = \mathbf{0.05y \div W}$

#### (3) Calculated by volumet of liquid samples

Superoxide Anion content (µmol/mL) =  $y \times 10^{-3} = \mathbf{y}$

Superoxide Anion production rate (µmol/min/mL) =  $y \div T \times 10^{-3} = \mathbf{0.05y}$

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

## Typical Data

Typical standard curve:

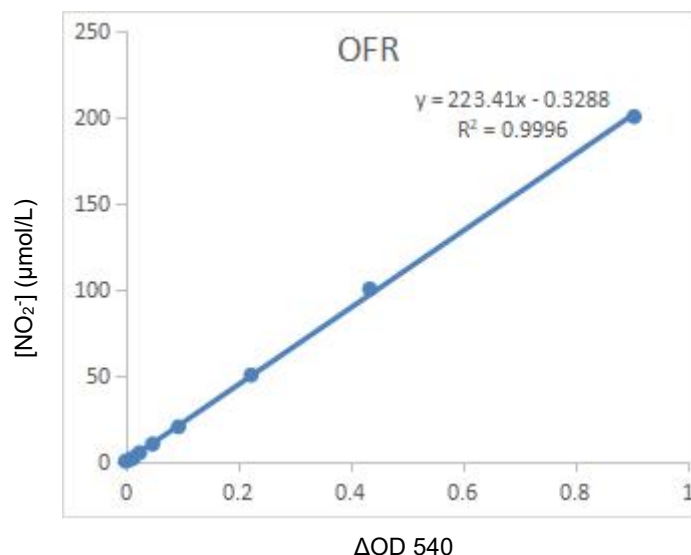


Figure 1. Standard curve for Superoxide Anion.

## Recommended Products

Catalog No.	Product Name
KTB1050	CheKine™ Micro Lipid Peroxidation (MDA) Assay Kit
KTB1041	CheKine™ Micro Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) Assay Kit
KTB1310	CheKine™ Micro Glucose Oxidase Activity (GOD) Assay Kit
KTB1070	CheKine™ Micro Xanthine Oxidase (XO) Activity Assay Kit
KTB1200	CheKine™ Micro Protein Carbonyl Assay Kit

## Disclaimer

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