Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Superoxide Anion Assay Kit

Cat #: KTB1210 Size: 48 T/48 S 96 T/96 S

	Micro Superoxide Anion Assay Kit		
REF	**Cat #:** KTB1210	LOT	**Lot #:** Refer to product label
	Detection range: 0.001-0.2 μmoL/mL		**sensitivity:** 0.001 μmoL/mL
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells		
	Storage: Stored at 4℃ 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 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	80 mL	80 mL×2	4℃
Reagent Ⅰ	6 mL	12 mL	4℃
Reagent Ⅱ	9 mL	18 mL	4℃, protected from light
Reagent Ⅲ	9 mL	18 mL	4℃, protected from light
$NaNO_2$ Standard (10 mmoL/L)	0.5 mL	0.5 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

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℃.

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.

Note: Reagent Ⅰ is toxic and has a pungent odor, Reagent Ⅱ is toxic, so it is recommended to experiment in a fume hood.

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:

Num.	Volume of 200 μmol/L $NaNO_2$ Standard (μL)	Volume of Extraction Buffer (μL)	Concentration (μmoL/L)
Std.1	200	0	200
Std.2	200 μL of Std.1 (200 μmoL/L)	200	100
Std.3	200 μL of Std.2 (100 μmoL/L)	200	50
Std.4	200 μL of Std.3 (50 μmoL/L)	200	25
Std.5	200 μL of Std.4 (25 μmoL/L)	200	12.5
Std.6	200 μL of Std.5 (12.5 μmoL/L)	200	6.25
Std.7	200 μL of Std.6 (6.25 μmoL/L)	200	3.125

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. After the sample is prepared, test immediately. Do not save the sample for a long time, so as not to affect the test results.

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℃. 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℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells: Collect 5×10^6 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℃. Use supernatant for assay, and place it on ice to be tested.
4. Serum, Plasma: 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
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 g for 5 min at 25℃, carefully add 200 μL of the upper aqueous phase into 96-well plate or microglass cuvette, measure the absorbance value at 540 nm 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 tube to eliminate the influence of NO_2^- existing in the sample itself. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.005, increase the sample quantity appropriately. If ΔA_{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 $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Bring the ΔA_{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)$=2\times y\times V_{Sample}\div(V_{Sample}\times Cpr)\times 10^{-3}$**$=0.002y\div Cpr$**

Superoxide Anion production rate (μmol/ min/mg prot) $=2\times y\times V_{Sample}\div(V_{Sample}\times Cpr)\div T\times 10^{-3}$**$=0.0001y\div Cpr$**

(2) Calculated by fresh weight of samples

Superoxide Anion content (μmol/g fresh weight)$=2\times y\times V_{Sample}\div(V_{Sample}\div V_{Sample\ Total}\times W)\times 10^{-3}$**$=0.002y\div W$**

Superoxide Anion production rate (μmol/min/g fresh weight)$=2\times y\times V_{Sample}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T\times 10^{-3}$**$=0.0001y\div W$**

(3) Calculated by volumet of liquid samples

Superoxide Anion content (μmol/mL)$=2\times y\times 10^{-3}$**$=0.002y$**

Superoxide Anion production rate (μmol/min/mL)$=2\times y\div T\times 10^{-3}$**$=0.0001y$**

(4) Calculated by cell samples

Superoxide Anion content (μmol/10^4)$=2\times y\times V_{Sample}\div(V_{Sample}\div V_{Sample\ Total}\times N)\times 10^{-3}$**$=0.002y\div N$**

Superoxide Anion production rate (μmol/10^4)$=2\times y\times V_{Sample}\div(V_{Sample}\div V_{Sample\ Total}\times N)\div T\times 10^{-3}$**$=0.0001y\div N$**

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 mL=10^{-3} L; N: Cell number, 500; 2:2 molecule $O2^-$ involved in the reaction to generate 1 molecule $NO2^-$.

Typical Data

Typical standard curve:

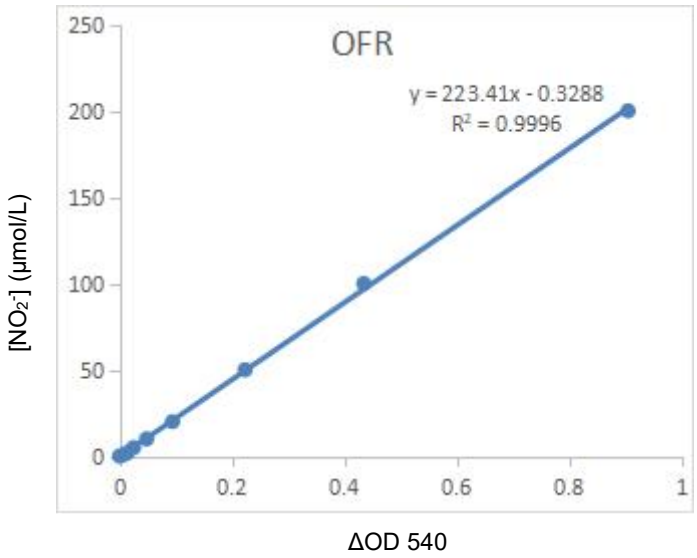

Figure 1. Standard curve for Superoxide Anion.

Recommended Products

Catalog No.	Product Name
KTB1050	CheKine™ Micro Lipid Peroxidation (MDA) Assay Kit
KTB1041	CheKine™ Micro Hydrogen Peroxide (H_2O_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. For your safety and health, please wear a lab coat and disposable gloves.