EZMeta™ Superoxide Anion Colorimetric Assay Kit

Cat #: D-AKC1210

Size: 48T / 96T

Storage: Stored at 4°C for 6 months, protected from light

Product Information

Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant

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°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
$NaNO_2$ 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.

ReagentⅢ: 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 $NaNO_2$ 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	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 5×10^6 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°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 EZMeta™ Superoxide Anion Colorimetric Assay Kit, 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°C water bath for 20 min				
Reagent Ⅱ	60	60	60	60
ReagentⅢ	60	60	60	60
Mix well, incubate in 37°C water bath for 20 min				
Trichloromethane	100	100	100	100
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. $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: Each sample needs to set up a control tube to eliminate the influence of NO_2^- existing in the sample itself, 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 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 y=kx+b. 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)=$y \times V_{Sample} \div (V_{Sample} \times Cpr) \times 10^{-3}$**=y÷Cpr**

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

(2) Calculated by fresh weight of samples

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

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

(3) Calculated by volumet of liquid samples

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

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

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.

Typical Data

Typical standard curve:

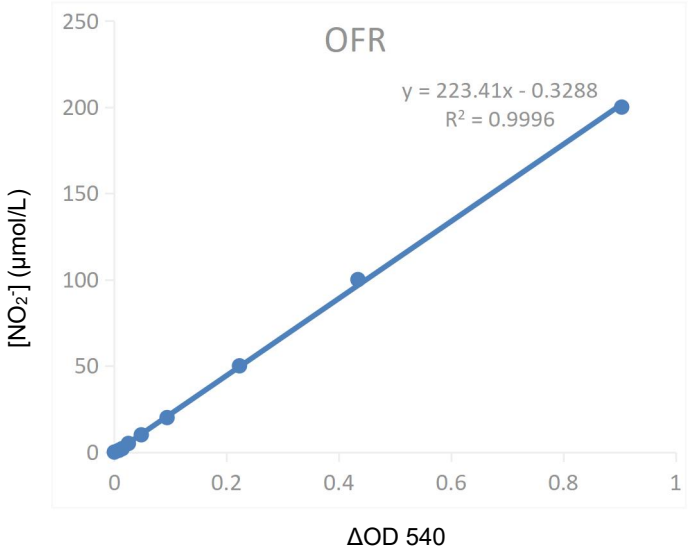

Figure 1. Standard curve for Superoxide Anion.

Disclaimer

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