(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Inhibition and produce superoxide anion Assay Kit

Catalog No.: BC00177

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Inhibition and produce superoxide anion Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell
Assay Type	Quantitative
Detection Instrument	Spectrophotometer, Microplate reader (550 nm)

Principle

In the simulated xanthine-xanthine oxidase reaction system in vivo, superoxide anion free radicals are generated. After adding an electron transfer substance and a Gress reagent, the reaction system exhibits a purple-red color, whose absorbance can be measured with a spectrophotometer.

When the tested sample contains an inhibitor of superoxide anion free radicals, the absorbance of the assay tube is lower than that of the control tube. Conversely, if the tested sample contains substances that generate superoxide anion free radicals, the absorbance of the assay tube is higher than that of the control tube.

Using vitamin C as a standard, the effect of the tested sample on superoxide anion free radicals can be calculated.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	5 mL	Store at 4 °C. Crystals may form in cold weather or when stored in a refrigerator. Dissolve in a 37 °C water bath before use. When in use, mix with double-distilled water at a ratio of 1:9 to prepare Reagent 1 working solution, which is also stored at 4 °C.
Reagent 2	Liquid	5 mL	Store at 4 °C
Reagent 3	Liquid	5 mL	Store at 4 °C

Reagent 4	Stock solution	350 µL	Store the stock solution at -20 °C and the diluent at 4 °C. Before use, prepare the Reagent 4 working solution: by mixing stock solution and diluent at a ratio of 1:14. Keep it on ice and use freshly prepared.
	Diluent	5 mL	
Reagent 5	Powder	1 vial	Add 37.5 mL of double-distilled water before use, and heat to 70-80 °C to dissolve. Store at 4 °C away from light after preparation.
Reagent 6	Powder	1 vial	Add 37.5 mL of double-distilled water before use, and heat to 70-80 °C to dissolve. Store at 4 °C away from light after preparation.
Reagent 7	Vitamin C standard	4 vials	When in use, add double-distilled water to one tube of VC standard and dilute to a final volume of 5 mL to prepare the VC standard stock solution (to be used within the same day after preparation). Then take 1 mL of this stock solution, add 4 mL of double-distilled water (5-fold dilution) to prepare a 0.15 mg/mL VC standard working solution. Prepare fresh before use.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Note: Preparation of color developer: Mix Reagent 5 solution, Reagent 6 solution and glacial acetic acid at a volume ratio of 3:3:2. Store at 4 °C away from light.

Storage

Store as instructed, valid for 6 months.

Preparation

Including a spectrophotometer with a 550 nm wavelength and 1 cm pathlength cuvettes (or a microplate reader and 96-well plates), a 37 °C water bath or incubator, an electric furnace (for heating during reagent preparation), beakers and glass rods, a desktop low-speed centrifuge, pipettes of various sizes, double-distilled water, glacial acetic acid (analytical grade), physiological saline (0.9%) or PBS (0.1 M), a vortex mixer, test tubes or centrifuge tubes, and protein assay reagents.

- **Sample handling**

1. Serum (plasma) samples: Hemolysis must be avoided during blood collection. Serum (plasma) can be used directly. Samples are preferably stored at 4°C and assayed on the same day. If not possible, they may be frozen below 0 °C; the lower the temperature, the longer the storage time.
2. Tissue samples: Accurately weigh the tissue to be tested. Add 9 volumes of physiological saline at a ratio of weight (g): volume (mL) = 1:9. Prepare a 10% tissue homogenate by mechanical homogenization in an ice-water bath. Centrifuge at 2500 rpm for 10 minutes, and collect the supernatant for assay. (A portion of the supernatant may be taken for protein concentration determination; protein quantification kits are available from our company.)
3. Cell samples: Collect cells (for adherent cells, digest with trypsin or scrape off with a cell scraper, transfer to a centrifuge tube, centrifuge at 1000-2000 rpm for 5 minutes, discard the supernatant and retain the pellet; for suspension cells, collect the pellet by direct centrifugation after transfer). Wash once or twice with 0.5-1 mL of PBS, centrifuge at 1000-2000 rpm to collect the cell pellet, then resuspend the cells in 0.3-0.5 mL of 0.1 M isotonic PBS buffer (pH 7.4). Lyse the cells by ultrasonication or manual grinding, and use the resulting cell lysate for testing (if obvious particles or flocs exist in the lysate, centrifuge at 2000 rpm for 10 minutes and use the supernatant).

Operation process

	Blank tube	Standard tube	Assay tube
Reagent 1 Working Solution (mL)	1.0	1.0	1.0
double-distilled water (mL)	0.05		
0.15 mg/mL VC standard solution(mL)		0.05	

Sample (mL)			0.05
Reagent 2 (mL)	0.1	0.1	0.1
Reagent 3 (mL)	0.1	0.1	0.1
Reagent 4 Working Solution (mL)	0.1	0.1	0.1
Mix thoroughly using a vortex mixer, then incubate in a 37°C constant-temperature water bath for 40 minutes.			
Color developer (mL)	2.0	2.0	2.0
Mix well and allow to stand at room temperature for 10 minutes. Zero the instrument with double-distilled water, then measure absorbance at 550 nm using a 1 cm pathlength cuvette.			

Note: Under the specified substrate concentration in the reaction system, different substances exhibit varying capacities to scavenge superoxide anions. According to the Lambert-Beer Law, the sample concentration should be neither too high nor too low, otherwise the results will be affected. Therefore, a preliminary test must be performed before the formal experiment to determine the optimal sampling concentration.

Calculation

1. Formula and calculation of anti-superoxide anion radical activity units in liquid samples such as serum (plasma)

   Definition: One activity unit (U) is defined as the change in superoxide anion radical inhibition by per liter of serum (plasma) or liquid sample in the reaction system at 37 °C for 40 minutes, equivalent to that inhibited by 1 mg of vitamin C.

   Calculation formula:

$$\text{Anti} - \text{superoxide anion capacity (U/L)} = \frac{A_{Blank} - A_{Assay}}{A_{Blank} - A_{Standard}} \times C_{Standard} \times 1000 \times N$$

2. Formula and calculation of anti-superoxide anion radical activity units in tissues and cells

   Definition: In the reaction system, one unit of activity (U) is defined as the change in superoxide anion radical inhibition by per gram of tissue (cellular) protein at 37 °C for 40

minutes, equivalent to that inhibited by 1 mg of vitamin C.

Calculation formula:

$$\text{Anti} - \text{superoxide anion capacity (U/g)} = \frac{A_{Blank} - A_{Assay}}{A_{Blank} - A_{Standard}} \times C_{Standard} \times 1000 \div \text{Cpr}$$

Annotation:

$C_{Standard}$: Standard solution concentration, 0.15 mg/mL

1000: Unit conversion, mL → L

N: Dilution factor of the sample before testing

Cpr: Protein concentration at the corresponding tissue sample concentration

Precautions

1. Wear a lab coat and disposable gloves during operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. Under the specified substrate concentration in the reaction system, different substances exhibit different anti-superoxide anion capacities. The sampling concentration should be neither too high nor too low, otherwise it will affect the results. Therefore, a preliminary test must be performed before the formal experiment to determine the optimal sampling concentration.
4. General lipid-containing specimens can be assayed according to this procedure, and interference from lipids on the determination should be excluded.