# Superoxide dismutase Assay Kit

## Cat#:orb1500005 (manual)

**Microassay**

**Size: 100T/96S**

**Product composition and storage conditions :**

| No. | Specifications | Storage Conditions |
|---|---|---|
| orb1500005- A | 25mL×1 | 4 ℃ ; |
| orb1500005- B | 2mL×1 | 4 ℃ ; |
| orb1500005- C | 15mL×1 | 4 ℃ ; |
| orb1500005- D | 35uL×1 | -20 ℃ ; |
| orb1500005- E | 40mL×1 | 4 °C away from light; |
| orb1500005- F | 40mL×1 | 4 °C away from light. |

## Introduction :

**Significance:** Superoxide dismutase (SOD; EC 1.15.1.1) is widely found in animals, plants, microorganisms and cultured cells. It can catalyze the dissimilation of superoxide anion to produce $H_2O_2$ and $O_2$. SOD is not only a superoxide anion scavenging enzyme, but also a major $H_2O_2$-producing enzyme, which plays an important role in biological antioxidant system.

**Principle:** Superoxide anion ($O^{2-}$) is produced by xanthine and Xanthine oxidase reaction system. $O^{2-}$ can reduce hydroxylamine to nitrite, which appears purplish red under the action of color reagent and is absorbed at 550 nm, the darker the color of the reaction solution, the lower the activity of SOD and the higher the activity of SOD.

## Own supplies :

Visible spectrophotometer or microplate reader, centrifuge, adjustable pipette , water bath, mortar, ice, distilled water

## Reagent preparation:

orb1500005-D working solution: Prepare according to the ratio of orb1500005-D : orb1500005-A=1:60 when using, and prepare according to the specific dosage.

## Sample processing :

1. Serum (plasma ,) cell culture fluid and other liquid samples: direct detection

2. Preparation of tissue samples:

**Animal tissue:** According to the ratio of tissue mass (g): normal saline is 1: 9, add 9 times of volume of homogenate medium,

prepare homogenate under the condition of ice bath, centrifuge at 2500-3000 rpm for 10 minutes, and take the supernatant for testing.

**Plant Tissue:** According to the ratio of tissue mass (g): 0.1mol/L PBS (ml) is 1:4, add 4 times the volume of homogenate medium, prepare homogenate under ice bath conditions, 3500-4000 rpm for 10 minutes, and take supernatant for testing.

## Measurement steps:

1. Preheat the visible spectrophotometer or microplate reader for at least 30 minutes, adjust the wavelength to 550nm .
2. Add the following reagents in sequence to the EP tube :

| Reagent name | Measuring tube ( uL ) | Control tube ( uL ) |
|---|---|---|
| orb1500005- A | 200 | 200 |
| Sample | 2 | |
| Distilled water | | 2 |
| orb1500005- B | 20 | 20 |
| orb1500005- C | 120 | 120 |
| orb1500005-D working solution | 20 | 20 |
| Mix thoroughly and place in 37 °C water bath for 30 minutes. | | |
| orb1500005- E | 400 | 400 |
| orb1500005- F | 400 | 400 |
| Mix thoroughly, place at room temperature for 15 minutes, and measure the absorbance value A of each tube at 550 nm. | | |

## SOD Enzyme activity calculation :

1. **Calculation of inhibition rate :**

Inhibition rate = (A Control tube -A measuring tube ) ÷A control tube

Try to keep the inhibition rate of the sample between 0.15-0.60 within the range. If the calculated inhibition rate is outside this range, it is usually necessary to adjust the sample volume and re-measure. If the measured inhibition rate is high, the sample needs to be appropriately diluted with the extraction solution; if the measured inhibition rate is low, the sample to be tested needs to be prepared again with a higher concentration.

2. **SOD Enzyme activity calculation :**

(1) **Calculation of SOD activity of serum (plasma), cell culture medium and other liquid samples:**

Definition of SOD enzyme activity: The amount of SOD corresponding to 50% SOD inhibition per milliliter of reaction solution is one SOD activity unit (U).

SOD Activity (U/mL) = Inhibition rate ÷ 50% × sample dilution times × total volume of reaction system ÷ sample detection volume

(2) **SOD activity calculation of tissue samples:**

Definition of SOD enzyme activity: The amount of SOD corresponding to 50% SOD inhibition per milligram of tissue protein in 1 ml of reaction solution is one SOD activity unit (U) .

SOD Activity (U/mg) = Inhibition rate ÷ 50% × total volume of reaction system ÷ sample detection volume ÷ sample protein concentration ( mg/ml )