A001-1 100/50 Tests

Superoxide Dismutase (SOD) Assay Kit

Organism Species: *Pan-species* (General)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition

[INTENDED USE]

This assay kit is designed to measure the activity of superoxide dismutase based on the xanthine oxidase method and is suitable for the samples like serum/plasma, cerebrospinal fluid, pleural effusion, peritoneal fluid, renal dialysis fluid, urine, red blood cells, white blood cells, platelets, cultured myocardium cells, cultured neoplasm, and other cells or subcellular components (mitochondria or microsome etc.). Also, researchers can use this kit for the measurement of samples such as micro-organisms, drugs, food, beverages and cosmetics.

Superoxide dismutase plays an important role in the balance of oxidation and anti-oxidation in vivo and can eliminate the superoxide anions in vivo to avoid cell damage by the superoxide anions.

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity(100/50T)	Reagents	Quantity(100/50T)
Reagent I	1×10ml/1×5ml	Reagent IV	2×350μl/1×350μl
Reagent II	1×10ml/1×5ml	Reagent V	1/1
Reagent III	1×10ml/1×5ml	Reagent VI	1/1
Reagent IV Diluent	1×10ml/1×5ml	Instruction manual	1/1

[MATERIALS REQUIRED BUT NOT SUPPLIED]

1. Spectrophotometer to measure the absorbance at 550 nm
2. Thermostatted water bath or gas bath (at 37℃)
3. Tabletop centrifuge
4. Air displacement pipettes
5. Double distilled water (DDW)
6. Acetic acid (analytic grade, ≥99.5%)

[STORAGE OF THE KITS]

1. Reagent I: Stock Buffer. Can be stored at 4℃ for 6 months. The solute may be crystallized at low temperature and under such circumstance, the precipitant should be warmed to dissolve before use.
2. Reagent II: Substrate Solution. Can be stored at 4℃ for 6 months.
3. Reagent III: Matrix. Can be stored at 4℃ for 6 months.
4. Reagent IV: Stock Enzyme Solution. Can be stored at -20℃ for 6 months.
5. Reagent IV Diluent: Can be stored at 4℃ for 6 months.
6. Reagent V: Powder. Can be stored at 4℃ for 6 months.

7. Reagent VI: Powder. Can be stored at 4℃ for 6 months.

[REAGENT PREPARATION]

1. **Reagent I Solution Preparation**：Dilute the stock buffer with double distilled water (DDW) to the final volume of 100ml(100T)/50ml(50T) and the buffer solution can be preserved at 4℃ for 3 months.
2. **Reagent IV Solution Preparation**：Dilute the Reagent IV(stock enzyme solution) using Reagent IV diluent with the ratio 1:14 and it is recommended to prepare the exact amount needed. The Reagent IV Solution should be placed at room temperature for 30 min before usage.
3. **Reagent V Solution Preparation**：Dilute with 70-80℃ DDW to a final volume of 75ml(100T)/37.5ml(50T)，the Reagent V solution can be preserved at 4℃ for 6 months without light struck.
4. **Reagent VI Solution Preparation**：Dilute with DDW to a final volume of 75ml(100T)/37.5ml(50T)， the Reagent VI solution can be preserved at 4℃ for 6 months without light struck.
5. **Preparation of Chromogenic Agent**：Blend the reagent V solution , reagent VI solution and acetic acid with the ratio 3:3:2 and the chromogenic agent can be preserved at 4℃ for 3 months.

Note:

1. Acetic acid used is analytical reagent with purity≥99.5%.
2. All ratios are expressed as volume ratios.

[SAMPLE PREPARATION]

1. Red Blood Cells:

Pretreatment: The pretreatment of red blood cells include a 50 μL whole blood being separated by centrifugation at 500-1000rpm for 10 min, and the pellets being hemolyzed by distilled water and hemoglobin being extracted by 0.2mL distilled water, 0.1mL 95% ethanol and 0.1mL chloroform.

2. Serum from Hyperlipemia Patients:

Pretreatment:

Serum from mild hypercholesterolemia patients: Serum can be diluted with the same amount of saline before the measurement.

Serum from moderate hypercholesterolemia patients: Serum should be diluted with the same amount of saline and half amount of 95% ethanol before the measurement.

Serum from severe hypercholesterolemia patients: Take 50 μL serum and blended with 200 μL saline and 150 μL ethanol. Then add 150 μL chloroform and centrifuge at 3500 rpm for 10 min. Extract the supernatant for measurement.

[ASSAY PROCEDURE]

1. Total-SOD Pre-measurement for Optimum Quantity of Sample Used

Extract 10 μl, 30 μl and 50 μl sample fluid respectively and measure the absorbance with the components and procedures listed below. Calculate the inhibition rate of each sample quantity with one single reference.

Inhibition rate= $(A_{Reference} - A_{Sample}) / A_{Reference}$

The sample quantity with the inhibition rate within 45%-50% should be used for the rest of the measurement.

Note: Were the results too high, the samples can be diluted for another pre-measurement.

2. Measurement

① **Sample:**serum/plasma, cerebrospinal fluid, pleural effusion, peritoneal fluid, renal dialysis fluid, urine, red blood cells, white blood cells, platelets, cultured myocardium cells, cultured neoplasm, and other cells or subcellular components (mitochondria or microsome etc.).

Operation table:

Reagent (ml)	Total SOD Reference (A)	Total-SOD (B)
Reagent I solution	1.0	1.0
DDW	a	
Sample		a
Reagent II	0.1	0.1
Reagent III	0.1	0.1
Reagent IV solution	0.1	0.1
Vortex till fully mixed and incubate at 37° C for 40 min		
Chromogenic Agent	2	2
Mix thoroughly, and set aside the mixture at room temperature for 10 min. After that, zero the spectrophotometer with DDW and measure the absorbance at 550nm with 1cm light path.		

② **Serum from Hyperlipemia Patients**

Operation table:

Reagent (ml)	Total SOD Reference (A)	Total-SOD (B)
Buffer Solution	1.0	1.0
DDW		
Sample		a
Substrate Solution	0.1	0.1
Stroma Solution	0.1	0.1
Enzyme Solution	0.1	0.1
Vortex till Fully Mixed and Warm at 37℃ for 40 min		
Chromogenic Agent	2	2
Sample	a	
Mix thoroughly, and set aside the mixture at room temperature for 10 min. After that, zero the spectrophotometer with DDW and measure the absorbance at 550nm with 1cm light path.		

Note:

1. a is an arbitrary number and represents the optimum volume determined at the previous step.
2. The procedures should be strictly followed and different types of reagents cannot be mixed before the measurement.

[TEST PRINCIPLE]

Super oxide anions generated as the byproduct of Xanthine Oxidase catalyzed Xanthine oxidation and the anion can oxidize hydroxylamine to nitrite which appears to be amaranth purple in the presence of chromogenic agent and thus the absorbance at certain wavelength can be detected by spectrophotometer. The presence of SOD in the system specifically inhibits the oxidization caused by super oxide anions and because of it, fewer nitrite

anions are generated. This would lower the absorbance of the sample tube compared to the reference tube without SOD and the SOD activity can be calculated with the formula given.

There are two types of SODs in higher animal cells: CuZn-SOD and Mn-SOD with total SOD activity equals to the sum of the activities of both type. Pretreatment should be done to remove the activity of Mn-SOD in sample while retain its original CuZn-SOD activity.

[CALCULATION OF RESULTS]

1. SOD Activity Calculation for Fluids：

① Definition

One SOD activity unit is defined as 1ml fluid among which the inhibition rate of SOD is 50%.

② Calculation Formula

$$\underset{(U/ml)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times \frac{V_{Reaction\ \ Total}}{V_{Sample\ \ Fluid}} \times CoD$$

Note: CoD represents the coefficient of dilution before the measurement.

③ Example

30μl untreated plasma was measured and the absorbance values of the reference and sample tube are 0.476 and 0.281 respectively.

$$\underset{(U/ml)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times \frac{V_{Reaction\ \ Total}}{V_{Sample\ \ Fluid}} \times CoD$$

$$= (\frac{0.476 - 0.281}{0.476}) \div 50\% \times \frac{3.33ml}{0.03ml} \times 1$$

$$= 90.95U/ml$$

Culture medium 100μL was measured and the absorbance values of the reference and sample tube are 0.473 and 0.312 respectively.

$$\underset{(U/ml)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times \frac{V_{Reaction\ \ Total}}{V_{Sample\ \ Fluid}} \times CoD$$

$$= (\frac{0.473 - 0.312}{0.473}) \div 50\% \times \frac{3.40ml}{0.10ml} \times 1$$

$$= 23.15U/ml$$

2. SOD Activity Calculation for Tissue homogenate:

① Definition

One SOD activity unit is defined as 1mg tissue protein among which the inhibition rate of SOD is 50%.

② Calculation Formula

$$\underset{(U/mg)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times \frac{V_{Reaction\ \ Total}}{V_{Sample\ \ Fluid}} \div \frac{C_{protein}}{mg/ml}$$

③ Example

1% mouse hepatic tissue homogenate was prepared and measured and the absorbance values were 0.510 and 0.242 respectively. Also, the protein concentration of the homogenate was 1.075 mg/ml.

$$\underset{(U/mg)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times \frac{V_{Reaction\ \ Total}}{V_{Sample\ \ Fluid}} \div \frac{C_{protein}}{mg/ml}$$

$$= (\frac{0.510 - 0.242}{0.510}) \div 50\% \times \frac{3.33ml}{0.03ml} \div 1.075mg/ml$$

$$= 108.5U/mg$$

Note: For plant tissues, the definition of the SOD activity unit can be re-defined as 1 g tissue among which the inhibition rate of SOD is 50%. And for this definition, calculation formula is shown below:

$$\underset{(U/mg)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times \frac{V_{Reaction\ \ Total}}{V_{Sample\ \ Fluid}} \div \frac{W_{tissue/g}}{V_{homogenate/ml}}$$

50μL 10% Arabidopsis leaf homogenate was measured and the absorbance values were 0.540 and 0.274 respectively.

$$\underset{(U/mg)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times \frac{V_{Reaction\ \ Total}}{V_{Sample\ \ Fluid}} \div \frac{W_{tissue/g}}{V_{homogenate/ml}}$$

$$= (\frac{0.540 - 0.274}{0.540}) \div 50\% \times \frac{3.35ml}{0.05ml} \div \frac{0.1g}{0.9ml}$$

$$= 594.1U/mg$$

3. SOD Activity Calculation for Red Blood Cells:

① Definition

One SOD activity unit is defined as 1g hemoglobin in 1 ml solution among which the inhibition rate of SOD is 50%.

② Calculation Formula

$$\underset{(U/gHb)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times V_{Reaction\ \ Total} \times \frac{0.3ml}{V_{Sample}} \div V_{Blood} \div \frac{C_{Hemoglobin}}{mg/ml}$$

③ Example

10 μ L hemoglobin extracted by the method mentioned below was measured and the absorbance values were 0.465 and 0.293 respectively. And the hemoglobin concentration in whole blood is 105mg/L (0.105mg/ml).

$$\underset{(U/gHb)}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times V_{Reaction\ \ Total} \times \frac{0.3ml}{V_{Sample}} \div V_{Blood} \div \frac{C_{Hemoglobin}}{mg/ml}$$

$$= (\frac{0.465 - 0.293}{0.465}) \div 50\% \times 3.31 \times \frac{0.3ml}{0.01} \div 0.05 \div 0.105$$

$$= 1.399 \times 10^4\ U/gHb$$

4. SOD Activity Calculation for Serum from Hyperlipemia Patients：

① Definition

One SOD activity unit is defined as 1ml fluid among which the inhibition rate of SOD is 50%.

② Calculation formula

$$\underset{U/ml}{Total-SOD\ Activity} = (\frac{OD_A - OD_B}{OD_A}) \div 50\% \times \frac{V_{Reaction\ \ Total}}{V_{Sample\ \ Fluid}} \times CoD$$

③ Example

Rabbit serum was pretreated and 150μL supernatant was measured with the absorbance values were 0.434 and

0.262 respectively.

$$Total-SOD\ \ Activity\ (\mathrm{U/ml}) = (\frac{\mathrm{OD_A}-\mathrm{OD_B}}{\mathrm{OD_A}}) \div 50\% \times \frac{\mathrm{V_{Reaction\ \ Total}}}{\mathrm{V_{Sample\ \ Fluid}}} \times \mathrm{CoD}$$

$$= (\frac{0.434-0.262}{0.434}) \div 50\% \times \frac{0.345}{0.15} \times 8$$

$$= 14.58\mathrm{U/ml}$$

[IMPORTANT NOTE]

1. All reagents should be prepared one day before the assay to ensure the complete dissolution of the reagents. Prepared reagents can be preserved at 4℃ for 3 months (expect the reagent IV) and should be placed at RT for 30 min before usage.
2. The incubation period is 40 min and can be prolonged to 45 min were the ambient temperature below 20℃.
3. Two blank tubes are required for the assay and the mean value should be used for the following calculation.
4. EDTA cannot be the anti-coagulant as it can react with metal ions in SOD to decrease the activity.