IK-113F-500 Superoxide Dismutase (SOD) Assay Kit

Size: 500 Tests

Product Description:

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion ($O_2^{\cdot-}$) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. In mammals, cytosolic SOD has a greenish color and consists of two subunits, one containing copper and the other zinc (Cu/Zn-SOD). Mitochondrial and bacterial SOD has a reddish-purple color and contains manganese (Mn-SOD). E. coli has Mn-SOD and SOD containing iron (Fe-SOD). Several direct and indirect methods have been developed to determine SOD activity. An indirect method using nitrotetrazolium blue is often used because of its convenience. However, there are several disadvantages to this method, such as poor water solubility of the formazan dye and its reaction with the reduced form of xanthine oxidase. Although cytochrome C is also commonly used for SOD activity detection, its reactivity with superoxide is too high to determine low levels of SOD activity.

SOD Assay Kit- allows a very convenient and highly sensitive SOD assay by utilizing the highly water-soluble tetrazolium salt, WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon reduction with a superoxide anion (Fig. 1). WST-1 is 70 times less reactive with superoxide anion than cytochrome C; therefore, highly sensitive SOD detection is possible and samples can be diluted with buffer to minimize background problems. WST-1 does not react with the reduced form of xanthine oxidase; therefore, even 100% inhibition with SOD is detectable. The rate of WST-1 reduction by superoxide anion is linearly related to the xanthine oxidase activity and is inhibited by SOD (see figure below). Therefore, the IC50 (50% inhibition concentration) of SOD or SOD-like materials can be determined using colorimetric methods.

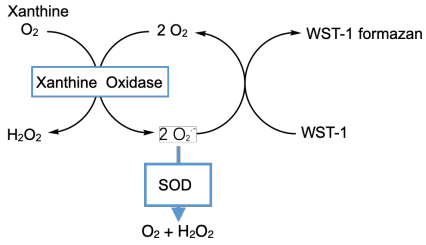

Fig. 1 SOD Inhibition assay mechanism. XO: xanthine oxidase。

Contents of the Kit:

SOD assay kit 500T		
Contents of the Kit	**Size**	**Storage**
Preparation liquid of SOD	250mL	-20°C avoid light 1year
SOD detection buffer	250ml	
WST-1 solution	4mL	
Enzyme solution	0.5ml	
Reaction starter (40X)	0.3ml	

Preparation of Various Sample Solution

Cells (Adherent cells: 9×10^6 cells, Leukocytes: 1.2 x107 cells)

1. Harvest cells with a scraper, centrifuge at 2,000 g for 10 min at 4ºC, and discard the supernatant.
2. Wash the cells with 1 ml PBS and centrifuge at 2,000 g for 10 min at 4ºC. Discard the supernatant. Repeat this step.
3. Break cells using the freeze-thaw method (-20ºC for 20 min, then 37ºC bath 10 min, repeat twice).
4. Add 1 ml PBS. If necessary, sonicate the cell lysate on an ice bath (60 W with 0.5 sec interval for 15 min).
5. Centrifuge at 10,000 g for 15 min at 4ºC.

Plant or Vegetable (200 mg)
1. Add 1 ml distilled water, and homogenize the sample using a homogenizer with beads.
2. Filter the homogenate with paper filter, and lyophilize the filtrate.
3. Measure the weight of the lyophilized sample, and dissolve with 0.1 M phosphate buffer (pH 7.4) to prepare sample solution.

Tissue (100 mg)
1. Wash the tissue with saline to remove as much blood as possible. Blot the tissue with paper towels and then measure its weight.
2. Add 400-900 µl sucrose buffer (0.25 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.4) and homogenize the sample using Teflon homogenizer. If necessary, sonicate the homogenized sample on an ice bath (60W with 0.5 second intervals for 15 min).
3. Centrifuge the homogenized sample at 10,000 g for 60 min at 4ºC, and transfer the supernatant to a new tube.

Tea (antioxidant activity detection)
1. Add 60 ml boiled water to 10 g of tea, and leave it for 2.5 min.
2. Filter the extract with paper filter and then filter again with a 0.45 µm membrane filter.
3. Dilute the filtrate with distilled water to prepare sample solution.

Erythrocytes or Plasma
1. Centrifuge 2-3 ml of anticoagulant-treated blood (such as heparin 10 U/ml final concentration) at 600 g for 10 min at 4°C.
2. Remove the supernatant and dilute it with saline to use as a plasma sample. Add saline to the pellet to prepare the same volume, and suspend the pellet.
3. Centrifuge the pellet suspension at 600 g for 10 min at 4ºC, and discard the supernatant.
4. Add the same volume of saline, and repeat Step 3 twice.
5. Suspend the pellet with 4 ml distilled water, then add 1 ml ethanol and 0.6 ml chloroform.
6. Shake the mixture vigorously with a shaker for 15 min at 4°C.
7. Centrifuge the mixture at 600 g for 10 min at 4ºC and transfer the upper water-ethanol phase to a new tube.
8. Mix 0.1 ml of the upper phase with 0.7 ml distilled water, and dilute with 0.25% ethanol to prepare sample solution.

Extracellular SOD (EC-SOD)
1. Prepare a 0.5 ml volume of Con A-sepharose column equilibrated with PBS.
2. Apply supernatant of a tissue homogenate on the column, and leave the column for 5 min at room temperature.
3. Add total 10 ml PBS to wash the column.
4. Add 1 ml of 0.5 M α-methylmannoside/PBS, and collect the eluate. Repeat 5 times.
5. Use the eluate for the SOD assay without dilution. If the SOD activity is high enough, dilute the eluate with PBS.

Wine (antioxidant activity detection)
1. Filter wine with a 0.45 µm membrane filter.
2. Dilute the filtrate with distilled water to prepare sample solution.

2. Preparations of Solutions

a) WST-1/ Enzyme working solution

The WST-1/Enzyme working solution is freshly prepared just before use according to the volume of 160uL for every reaction. The WST-1/Enzyme working solution is prepared by mixing well with the 151uL SOD detection buffer, 8uL WST-1 solution and 1uL Enzyme solution. The WST-1/Enzyme working solution should be stored at 4℃ or ice-bath. The following table is for the specific preparation methods.

Amount of test samples	1	10	20	50
SOD detection buffer (uL)	151	1510	3020	7550
WST-1 solution (uL)	8	80	160	400
Enzyme solution (uL)	1	10	20	50
WST-1/Enzyme working solution (uL)	160	1600	3200	8000

b) Preparation of reaction starter working solution:
The reaction starter working solution is freshly prepared just before use by mixing well with the 39 uL SOD detection buffer and 1uL Reaction starter (40X) and should be stored at 4℃ or ice-bath.

c) (Selected) Preparation SOD standards： The SOD standards are NOT provided by this kits. The SOD standard is diluted to the following concentration by our Preparation liquid of SOD：100U/ml，50U/ml，20U/ml，10U/ml，5U/ml，2U/ml，1U/ml . In subsequent tests, 20 μl can be taken respectively

3. Sample test:

a) On the 96-well plate the sample and blank are prepared according the following table. All the components are mixed well on the ice-bath.

	Sample	Blank1	Blank2	Blank3
Sample to be tested	20μl	-	-	20μl
SOD detection buffer (uL)	-	20μl	40μl	20μl
WST-1/Enzyme working solution	160μl	160μl	160μl	160μl
Reaction starter working solution	20μl	20μl	-	-

b) Incubate the plate at 37℃ for 30 minutes.
c) Read the absorbance at 450 nm using a microplate reader.
d) Calculate the SOD activity(inhibition rate %) using the following equation.

4. Calculate SOD in the test sample
a) Calculate the SOD activity(inhibition rate %) using the following equation.
SOD activity (inhibition rate %) = {[(Ablank 1-Ablank 2)-(Asample-Ablank3)]/(Ablank 1-Ablank 2)}x100
b) Definition of Unit(U)： One unit is defined as a point where a 20uL of sample solution gives 50% inhibition of a colorimetric reaction between WST-1 and superoxide anion.
* Unit definition differ from the unit definition of Cytochrome C assay.
c) SOD Calculate Unit(U)
1. Calculate a dilution ratio where the inhibition curve gives 50% inhibition.
2. SOD unit in original sample can be calculated by multiplying the dilution rate.

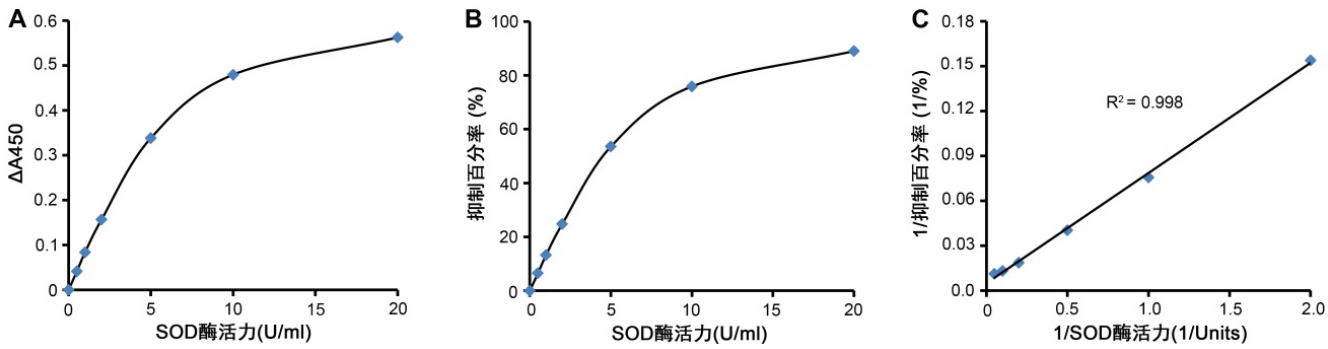

Fig2. SOD standards test。Fig A Y axis ΔA450 Incubate 30min，Absorbance difference between Blank 1 与 SOD standards. Fig B is the inhibition curve of SOD and Inhibition percentage ΔA450; FigC is the curve of 1/SOD and 1/ Inhibition percentage.

5 Interference
Reducing agents, such as ascorbic acids and reduced forms of glutathiones, interfere with the SOD assay. Table 2 shows the concentrations of materials that cause 10% increase in the O.D. value. If sample contains these materials, please dilute the sample to avoid the interfere.