Superoxide Dismutase (SOD) Activity Assay Kit with WST-1

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC5165

Size: 100T/48S

Components:

Extraction reagent: 60 mL×1. Storage at 2-8℃.

Reagent I: 5 mL×1. Storage at 2-8℃.

Reagent II: 25 μL×1. Storage at 2-8℃.

Preparation of Reagent II-working solution： Reagent **II** is diluted 50 times with sterile water before use according to the sample number and used up on the same day.

Reagent III: 4 mL×1. Storage at 2-8℃.

Reagent IV: 0.12 mL ×1. Storage at 2-8℃.

Preparation of Reagent TV-working solution： Reagent **IV** was diluted 10 times with distilled water before use according to the sample number and used up on the same day.

Product Description:

Superoxide dismutase (SOD, EC 1.15.1.1) is a kind of metalloenzyme widely found in organism. It is an important oxygen radical scavenger and can catalytic disproportionation of superoxide anion to form H_2O_2 and O_2. SOD is not only the superoxide anion scavenging enzyme, but also the main H_2O_2 producing enzyme, which plays an important role in the biological antioxidant system.

Superoxide anion (O_2^-) is produced by xanthine and xanthine oxidase reaction system. O_2^- can reduce tetrazolium-1(WST-1) to form a water-soluble yellow formazan dye, which has absorbance in 450 nm. SOD can remove O_2^- and inhibit the formation of the formazan dye. The darker the yellow color of the reaction solution, the lower the SOD activity. The lighter the yellow color of the reaction solution, the higher the activity of SOD.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, table centrifuge, water bath/constant temperature foster box, electronic balance, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/ homogenizer, ice and distilled water.

Operation steps:

I. Sample preparation:

1. Bacteria or cells: collect bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. It is suggested that 5 million of bacteria or cell amount with 1 mL of Extraction reagent. Splitting the bacteria or cell with ultrasonication (placed on ice, ultrasonic power 200W, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 g for 10 minutes at 4℃ to remove insoluble materials,

and take the supernatant on ice before testing.

2. Tissue: it is suggested that 0.1 g of tissue with 1 mL of Extraction reagent and fully homogenized on

ice bath. Centrifuge at 8000 g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

3. Serum (plasma) sample: detect sample directly. Centrifuge before detect if there are precipitation.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 450 nm, set spectrophotometer counter to zero with distilled water.

2. Keep Reagent I, Reagent III, Reagent IV-working solution in water bath for 5 minutes at 37℃(mammals) or 25℃ (other species).

3. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Control tube (C)	Blank tube (B1)	Blank tube (B2)
Sample	20	20	-	-
Reagent I	45	45	45	45
Reagent II-working solution	20	-	20	-
Reagent III	35	35	35	35
Distilled water	70	90	90	110
Reagent IV-working solution	10	10	10	10

Mix thoroughly and the mixture is incubated at 37℃ for 30 minutes. Add the mixture into ultra-micro cuvette/96 well flat-bottom plate, and detect the absorbance value of each tube at 450 nm. $\Delta A_T=A_T-A_C$, $\Delta A_B=A_{B1}-A_{B2}$. If there is precipitation at the bottom, mix thoroughly and then measure. Blank tubes need to test once or twice and every test tube need a contrast tube.

III. Calculation:

1. Inhibition percentage:

Inhibition percentage=$[\Delta A_B-\Delta A_T]\div\Delta A_B\times 100\%$

The inhibition percentage should be in 30%~70% (the value close to 50% will have a more accurate result). If the calculated inhibition percentage is less than 30% or more than 70%, it is usually necessary to adjust the sample addition amount and redetermine. If the percentage of inhibition is too high, the sample should be diluted properly. If the percentage of inhibition is too low, the sample should be reprepared with a higher concentration and reducing the distilled water volume at the same time.

2. Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the inhibition of 50% in the reaction system of the above xanthine oxidase.

3. Calculation

A. Serum (plasma) sample

SOD activity (U/mL)=$[P\div(1-P)\times Vrv]\div Vs\times F=10\times P\div(1-P)\times F$

B. Tissue, bacteria or cultured cells

a) Protein concentration:

SOD activity (U/mg prot)=[P÷(1-P)×Vrv]÷(Vs×Cpr)×F=10×P÷(1-P)÷Cpr×F

b) Sample weight

SOD activity (U/g weight)=[P÷(1-P)×Vrv]÷(W×Vs÷Vsv)×F=10×P÷(1-P)÷W×F

c) Bacteria or cell amount

SOD activity (U/10^4 cell)=[P÷(1-P)×Vrv]÷(500×Vs÷Vsv)×F=0.02×P÷(1-P)×F

Vrv: Total reaction volume, 0.2 mL;
Vs: Sample volume, 0.02 mL;
Vsv: Extraction volume, 1 mL;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight, g;
500: Total number of bacteria and cells, 5 million;
P: Inhibition percentage , %;
F: Sample dilution multiple.

Note:

1. The Sample and Reagent IIworking solution should be placed on ice when using.
2. When there are many samples, the working solution (including Reagent I, II working solution, III and distilled water) can be configured according to the table. Reagent IVworking solution must be added finally.

Experimental Examples:

1. 0.1 g of mice liver is added into 1 mL of Extraction reagent for homogenization. After the supernatant is taken and diluted 100 times, the operation is carried out according to the determination steps. The results with 96-well plates showed that $\Delta A_T = A_T - A_C$ = 0323-0.079=0.244, $\Delta A_B = A_{B1} - A_{B2}$ =0.507-0.077 =0.430. Inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\%$ = 43.26%, and the enzyme activity is calculated according to the sample mass.

SOD activity (U/g weight) = 10 × Inhibition percentage (1-Inhibition percentage) × W ×F = 7624.25 U/g weight.

References:

[1] Peskin A V, Winterbourn C C A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1) [J]. Clinica chimica acta, 2000, 293(1-2):157-166.

[2] Hou Z, Zhao L, Wang Y, et al. Purification and characterization of superoxide dismutases from sea buckthorn and chestnut rose[J]. Journal of food science, 2019, 84(4): 746-753.

Related Products:

BC0190/BC0195 Polyphenol Oxidase (PPO) Activity Assay Kit

BC0210/BC0215 Phenylalnine Ammonialyase (PAL) Activity Assay Kit

BC0200/BC0205 Catalase (CAT) Activity Assay Kit
BC0090/BC0095 Peroxidase (POD) Activity Assay Kit

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