Superoxide dismutase (SOD) Assay Kit

Cat No. SOD-M-50
Size: 50 Reactions
Store at 4°C

MOLEQULE-ON®

Components	Quantity
Extraction Reagent	60 ml
Reagent I	15 ml
Reagent II	160 µl
Reagent III	11 ml
Reagent IV	Powder
Reagent V*	2 ml

Description

Superoxide dismutase (SOD, EC 1.15.1.1) is a metalloenzyme widely present in living organisms. It is an important oxygen radical scavenger that catalyzes the deuteration of superoxide anions and produces H_2O_2 and O_2. SOD is not only a superoxide anion scavenging enzyme, but also a major enzyme producing H_2O_2, which plays an important role in the biological antioxidant system.
Superoxide anion (O_2^-) is produced by the xanthine and xanthine oxidase reaction system, and O_2^- reducible nitroblue tetrazolium produces blue formazan, which absorbs at 560 nm; SOD can remove O_2^-, thereby inhibiting The formation of formazan; the deeper the blue of the reaction solution, the lower the SOD activity, and the higher the activity.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, benchtop centrifuge, micropipette, cuvette, mortar, ice and distilled water.

Preparation:

**** Working solution V: add Reagent IV before use and dissolved by shaking.***

Protocol

I. Sample preparation:

1. Bacteria or cells:
Collect bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. Accordance ratio Bacteria or cell amount (10^4): Extraction reagent volume (ml) = 500~1000:1, suggested 5 million with 1ml Extraction reagent. Split bacteria and cell with ultrasonication (ice bath, 200W, work time 3s , interval 10s, repeat for 30 times). Centrifuge at 8000 rpm for 10 min at 4℃. Supernatant on the ice is used for test.

2. Tissue
Accordance ratio tissue weight (g): Extraction reagent volume (ml) = 1:5~10 (suggested 0.1g tissue with 1ml Extraction reagent). Fully grind on ice, centrifuge at 8000 g for 10 min at 4℃. Supernatant is used for test.

3. Serum (plasma) sample:
Detect sample directly.

II. Determination procedure:

1. Preheat the spectrophotometer 30min, adjust wavelength to 560 nm, set zero with distilled water.

2. Keep Reagent I, Reagent Ⅲ, Reagent Ⅴ in water bath for more than 5 min: 37℃ (mammal cell) , 25℃ (other species).
3. Add reagents with the following list:

(μL)	Test tube (A_T)	Control tube (A_C)	Blank tube(A_{B1})	Blank tube(A_{B2})
Sample	90	90	-	-
Reagent Ⅰ	240	240	240	240
Reagent Ⅱ	6	-	6	-
Reagent Ⅲ	180	180	180	180
ddH_2O	480	486	570	576
Reagent Ⅴ	30	30	30	30

4. Mix thoroughly, incubate at 37℃ water bath for 30min, add to 1mL glass cuvette, and detect the absorbance value of each tube at 560 nm. ΔAT=AT-AC, ΔAB=AB1-AB2.

Note:

- During test, sample and Reagent Ⅱ should be placed on ice.
- For each sample please set one control, and 1-2 blank tube will be enough for test.
- When there are many samples, the working fluid (including Reagent, Ⅱ and Ⅲ) can be configured according to the table, and Reagent Ⅴ must be added last.
- After the reaction is completed, there may be precipitation, mix the solution before measurement.

Calculations

1. Inhibition percentage:

Inhibition percentage = $(\Delta A_B - \Delta A_T) \div \Delta A_B \times 100\%$

Try to make the percentage of inhibition of the sample within the range of 30-70%, the closer to 50%, the more accurate. If the calculated percent inhibition is less than 30% or greater than 70%, it is usually necessary to adjust the amount of the sample and re-measure. If the measured percentage of inhibition is high, the sample should be diluted appropriately; if the measured percentage of inhibition is low, the sample to be tested with a higher concentration needs to be re-prepared.

2. Unit definition: When the percentage of inhibition in the xanthine oxidase coupling reaction system is 50%, the SOD activity in the reaction system is defined as one enzyme activity unit.

3. SOD enzyme activity

A. Serum (plasma) sample

SOD(U/mL)=[P÷(1-P)×Vrv]÷Vs×N=11.4×P÷(1-P)×N

B. Tissue, bacteria or cultured cells

(i) Protein concentration:

SOD(U/mL)=[P÷(1-P)×Vrv]÷(Vs×Cpr)×N=11.4×P÷(1-P)÷Cpr×N

Recommand BCA Protein Assay Kit(PC0020) for protein concentration calculation.

(ii) Sample weight

SOD(U/g fresh weight)=[P÷(1-P)×Vrv]÷(W×Vs÷V_E)×N=11.4×P÷(1-P)÷W×N

(iii) Cell amount

SOD(U/10^4 cell)=[P÷(1-P)×Vrv]÷(500×Vs÷V_E)×N=0.0228×P÷(1-P)×N

Vrv: total reaction volume, 1.026mL;
Vs: the sample volume, 0.09mL;
V_E: the extraction volume, 1mL;
Cpr: sample protein concentration, mg/mL;
W: sample weight, g;
500: the total number of bacteria or cells, 5 million.
P: inhibition percentage, %;
N: Sample dilution multiple