Catalase (CAT) Activity Assay Kit

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

MOLEQULE-ON®

Components	Quantity
Extraction Reagent	60 ml
Reagent I	60 ml
Reagent II	320 µl

Description

Catalase (CAT) exists widely in animal, plant, microorganism and cultured cells, which is the main enzyme of clearing H_2O_2.
H_2O_2 has characteristic absorption peaks at 240nm, CAT resolves H_2O_2, make the absorbance of reagent at 240nm decreases , and the activity of CAT can be calculated according to the change rate of absorbance.

Reagents and Equipment Required but Not Provided:

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

Protocol

I. Sample preparation:

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

2. Tissue
Accordance ratio tissue weigh t(g): Extraction reagent volume (mL) = 1:5~10. Suggested 0.1g tissue with 1mL Extraction reagent. Fully grinding on ice, 8000g 4℃ centrifuge for 10 min. Supernatant is used for test.

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

II. Determination procedure:

1. Preheat the spectrophotometer 30 min, adjust wavelength to 240 nm, set zero with distilled water.
2. CAT working reagent: add 20 ml reagent I to 100µL reagent Ⅱ before use, mix thoroughly.
3. Preheat CAT working reagent in water bath: 37℃ (mammal cell), 25℃ (other species).
4. Add 1ml CAT working reagent in 1 ml cuvette, add 35µL sample, mix for 5s, detect absorbance A1 and A2 (after 1 min) at 240nm, calculate ΔA=A1-A2.

Calculations

1. Serum (plasma) sample

Unit definition: One unit of enzyme activity is defined as catalyzing 1nmol H_2O_2 per min in 1 ml serum(plasma).
CAT(U/mL)=[ΔA×V sv÷(ε×d)×109]÷V s÷T=678×ΔA

2. Tissue, bacteria or cultured cells

A. Protein concentration:

Unit definition: One unit of enzyme activity is defined as catalyzing 1nmol H_2O_2 per min in 1 mg protein.

CAT(U/mg prot)= [ΔA×Vrv÷(ε×d)×10^9]÷(V s×Cpr)÷T=678×ΔA÷Cpr

B. Sample weight

Unit definition: One unit of enzyme activity is defined as catalyzing 1nmol H_2O_2 per min in 1 g tissue.

CAT(U/g)=[ΔA×Vrv÷(ε×d)×10^9]÷(W×V s÷Vsv)÷T=678×ΔA÷W

C. Cell amount

Unit definition: One unit of enzyme activity is defined as catalyzing 1nmol H_2O_2 per min in 10000 cells.

CAT(U/10^4cell)= [ΔA×Vrv÷(ε×d)×10^9]÷(500×Vs÷Vsv)÷T=1.356×ΔA

Vrv: reaction total volume, 1.035×10^{-3} L;
ε: molar extinction coefficient, 4.36×10^4 L / mol /cm;
d: light path of cuvette, 1cm;
Vs: the sample volume,0.035 ml;
Vsv: the extraction volume, 1 ml;
T: Reaction time, 1 min
Cpr: Sample protein concentration, mg/mL;
W: sample weight, g;
500: the total number of bacteria and cells, 5 million.