# Catalase (CAT) Assay Kit

**Catalog No: SH0023**
**Method:** Colorimetric method
**Specification:** 100 tubes /50 samples

## Application
This kit can be used to measure CAT activity in animal serum, plasma and tissue homogenate samples.

## Detection principle
The reaction that catalase (CAT) decomposes $H_2O_2$ can be quickly stopped by ammonium molybdate. The residual $H_2O_2$ reacts with ammonium molybdate to generate a yellowish complex. CAT activity can be calculated by production of the yellowish complex at 405 nm.

## Kit components
**Reagent 1:** Buffer solution, 50 mL ×2 vials, store at 4℃ for 6months.
**Reagent 2:** Substrate, 10 mL ×1 vial, store at 4℃ for 6months.
Note: Incubate Reagent 1 and Reagent 2 at 37℃ for 30 min before use.
**Reagent 3:** Chromogenic agent, powder, 1 vial, store at 4℃ for 6 months. Dissolve the powder to 100 mL with double-distilled water. (If there is sediment in the bottom, please directly take the supernatant for test, it will not affect the result)
**Reagent 4:** Clarificant, 10 mL ×1 vial, store at 4℃ for 6 months. It is frozen when cold, please warm it with 37℃ water-bath until clear.

## Experimental instrument
Test tube, Micropipettor, Vortex mixer, Centrifuge, Spectrophotometer (405 nm)

## Sample preparation
1. **Serum/plasma or Cell supernatant:** Detect the sample directly. It need to centrifuge if serum precipitates existed, otherwise the result will be greatly affected. If the concentration is beyond the linear range, then dilute the sample with saline before detection. There is hemolysis in the serum sample, each sample should be needed a control, if not, you can use double-steamed water as the control.
2. **Tissue:** Mince the tissues to small pieces, then weighed and homogenized in PBS(0.01 M, pH 7.4) on ice, the volume of PBS (mL): the weight of the tissue (g) =9:1. The tissue homogenate is centrifuged at 3000 r/m for 15 min. Collect the supernatant for detect. Determine the concentration

of supernatant with BCA or CBB (Coomassie brilliant blue). If the sample is high-fat, each sample should be needed a control, if not, you can use double-steamed water as the control.

### Operation steps

| | Control tube | Sample tube |
|---|---|---|
| Sample (mL) | | a* |
| Reagent 1(preheated at 37℃) (mL) | 1.0 | 1.0 |
| Reagent 2 (preheated at 37℃) (mL) | 0.1 | 0.1 |
| Mix fully and react accurately at 37℃ for 1 min. | | |
| Reagent 3 (mL) | 1.0 | 1.0 |
| Reagent 4 (mL) | 0.1 | 0.1 |
| Serum (plasma) (mL)* | a* | |

Mix thoroughly, stand for 10 min at room temperature. Set to zero with double-distilled water and measure the OD values of each tube at 405 nm with 0.5 cm cuvette.

**Note: 1)** If there are no obvious hemolysis and blood fat for serum (plasma) samples, or there is no high- fat in tissue homogenate samples, * can be replaced by double-distilled water for control tube. Control tubes only need 1-2 for each experiment.

**2)** For Serum or plasma samples, a* is 0.1 mL. For Tissue homogenates, a* is 0.05 mL.

## Calculation of results

**1. Calculation formula of CAT activity in serum (plasma)**

Definition: The amount of CAT in 1 mL of serum or plasma that decompose 1 μmol $H_2O_2$ per second is defined as 1 unit.

CAT activity (*U/mL*)

$$= (OD_{Control} - OD_{Sample}) \times \frac{325^*}{60 \times \text{The volume of sample}} \times \text{Dilution factor of sample before tested}$$

**Note:** * 325 is the reciprocal of slope.

**2. Calculation formula of CAT activity in tissue homogenates**

Definition: The amount of CAT in 1 mg of tissue protein that decompose 1 μmol $H_2O_2$ per second is defined as 1 unit.

CAT activity (*U/mgprot*)

$$= (OD_{Control} - OD_{Sample}) \times \left(\frac{325^*}{60 \times \text{The volume of sample}}\right) \div \text{Protein concentration of tested sample } (mg/mL)$$

**Note:** *325 is reciprocal of slope.

## Technical parameter

1. The sensitivity of the kit is 0.045 U/mL.
2. The intra-assay CV is 3.1% and the inter-assay CV is 5.1%.
3. The recovery of the kit is 95.8%.
4. The detection range of the kit is 0.045-25.89 U/mL.

## Notes

1. Please operate strictly according to operation procedures.
2. The test tube can be prepared and labeled in advance. After incubating at 37 ℃ for 10 min, add samples and reagent 1, then incubate the test tube at 37 ℃ for 5 min.
3. Dilute the samples to the optimal concentration for detection if the CAT activity of samples exceed the detection range.
4. If serum and plasma samples are hemolysis, it should take control tube for each sample.
5. If tissue samples are high fat, it should take control tube for each sample.

This manual must be read attentively and completely before using this product.
May you have any problems, please contact our Technical Service Center for help.
Phone: 86-21-3100-7137
Email: save@bt-laboratory.com
Website: www.bt-laboratory.com