About This Kit

Catalase (CAT) is a ubiquitous antioxidant enzyme that is present in the peroxisomes of nearly all aerobic cells and provides cell protection against oxidative stress-induced damage by catalyzing the decomposition of H_2O_2 to water and oxygen (catalytic activity). Furthermore, CAT demonstrates peroxidatic activity in which low molecular weight alcohols can serve as electron donors. Although aliphatic alcohols are used as specific substrates by CAT, other enzymes with peroxidatic activity do not utilize these substrates.

TPR's Catalase Activity Assay Kit is a well-established tool for measuring the activity of CAT in **serum, plasma, tissue, cell lysates and other biological fluids**. This kit measures CAT activity via reaction of the CAT present in the sample with methanol in the presence of an optimal concentration of H_2O_2 to produce formaldehyde (Fig. 1). The formation of formaldehyde is colorimetrically determined by using a chromogen that turns aldehydes purple.

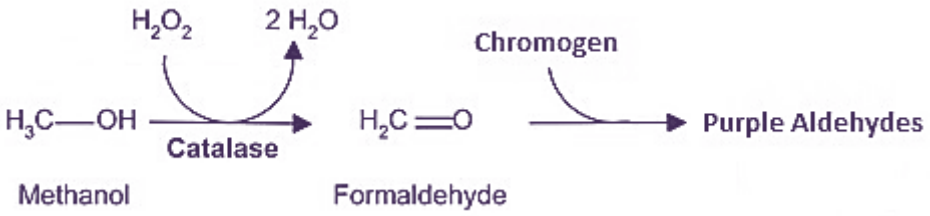


Fig. 1: Scheme of the CAT assay

Kit Components

Item Label	Item	48 Tests Kit	96 Tests Kit
Reagent 1 **(R1)**	Assay Buffer (10X)	3 mL	5 mL
Reagent 2 (**R2**)	Sample Buffer (10X)	6 mL	10 ml
Reagent 3 (**R3**)	Hydrogen Peroxide	300 µl	500 µl
Reagent 4 (**R4**)	Methanol	3 ml	5 ml
Reagent 5 (**R5**)	Stopper Reagent	2 ml	4 ml
Reagent 6 (**R6**)	Chromogenic Reagent	2 ml	4 ml
Reagent 7 (**R7**)	Oxidizer Reagent	1 ml	2 ml
Reagent 8 (**R8**)	Formaldehyde Standard	100 µl	200 µl
-	96 Well Microplate	1 Plate	1 Plate
-	Instruction Manual	1 Manual	1 Manual

Materials Required (Not Provided)

- Distilled water
- Disposables tubes
- PBS or 150 mM KCl (if using tissue samples)
- Dounce homogenizer (if using tissue)

Required Instrumentation

- Multiwell micro-plate reader (capable of measuring absorbance at 540 nm)
- Microfuge
- Pipettes (Multi-channel pipettes are preferred.)

Kit Performance

- **Precision:** Human catalase activity with 30 replicates on three different days showed intra and inter assay coefficient of variation 4.1% and 9.9% respectively.

Storage and Stability

- This kit will perform as specified if **stored at 4°C**.
- Use before the **expiration date** indicated on the box.

Sample Preparation

Important Note:

- All samples should be assayed immediately upon collection or stored at -80°C for up to 1-2 months.
- Always thaw samples on ice (Regulation of temperature at 4°C increases sensitivity and reproducibility).

PLASMA AND BLOOD SAMPLES

1. Collect whole blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge at 1,000 x g for 10 minutes at 4°C.
3. Carefully pipette off the plasma layer without disturbing the buffy layer, transfer to a fresh tube and place on ice.
4. Remove the buffy layer from the red cell pellet.
5. Re-suspend the erythrocytes in four times its volume of ice cold distilled water.
6. Centrifuge at 10,000 x g for 15 minutes at 4°C.
7. Collect the supernatant and transfer to a fresh tube and place on ice.

SERUM

1. Collect whole blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 3000 x g for 10 minutes at 4°C.
4. Carefully pipette off the yellow serum layer without disturbing the buffy layer and transfer to a new tube and place on ice.

Note: It is important to minimize hemolysis in plasma/serum samples.

TISSUE HOMOGENATES

1. Weight out the amount of tissue necessary for each assay (10-100 mg wet tissue).
2. Perfuse or rinse the tissue with PBS or 150 mM KCl, to remove any red blood cells and clots.
3. Homogenize the tissue in 1 ml ice cold PBS or 150 mM KCl.
4. Centrifuge at 14,000 x g for 5 minutes at 4°C.
5. Collect the supernatant and transfer to a fresh tube and place on ice.

Note: RIPA buffer (without SDS) supplemented with PMSF protease inhibitors can also be used as lysis buffer.

1. Harvest the amount of cells necessary for each assay (1 x 10^6 cells/ml recommended). For harvesting adherent cells, do not use proteolytic enzymes (like trypsin); rather use a rubber policeman.
2. Lyse cells by repeated cycles of freezing and thawing in ice cold PBS (pH 7.2-7.4).
3. Centrifuge at 14,000 x g for 5 minutes at 4°C.
4. Collect the supernatant and transfer to a fresh tube and place on ice.

Reagent Preparation

1) **Reagent 1 (R1)**: R1 vial contains 10X assay buffer. Dilute 2 ml of R1 with 18 ml distilled water (96 Tests kit) / 1 ml of R1 with 9 ml distilled water (48 Tests kit). The ready R1 reagent is stable for at least three months at 4°C.
2) **Reagent 2 (R2)**: R2 vial contains 10X sample buffer. Dilute 5 ml of R2 with 45 ml distilled water (96 Tests kit) / 3 ml of R1 with 27 ml distilled water (48 Tests kit). This ready R2 reagent is used to dilute samples and R8 reagent, and is stable for at least two months at 4°C.
3) **Reagent 3 (R3)**: R3 vial contains a 9.8 M solution of H_2O_2. Dilute 20 µl of R3 with 4.98 ml distilled water. The ready R3 reagent is stable for two hours at room temperature.
4) **Reagent 4 (R4)**: Ready to use.
5) **Reagent 5 (R5)**: Ready to use.
6) **Reagent 6 (R6)**: Ready to use.
7) **Reagent 7 (R7)**: Ready to use.

8) Standard Preparation:

Dilute 3.1 µl of Formaldehyde Standard (**R8**) with 9.997 ml of *Ready R2 reagent* to obtain a stock solution of 4.25 mM of Formaldehyde. Take seven clean microtubes and label them A-G. Add the amount of *Formaldehyde 4.25 mM stock solution* and *ready R2 reagent* to each tube as described below.

Tube	Formaldehyde (µl)	Ready R2 Reagent (µl)	Final Concentration (µM Formaldehyde)
A	0	1000	**0**
B	10	990	**5**
C	30	970	**15**
D	60	960	**30**
E	90	910	**45**
F	120	880	**60**
G	150	850	**75**

Things to Note

- Allow all reagents to equilibrate to room temperature before performing the assay.
- It is recommended that all samples and standards be assayed at least in duplicate.
- *The Catalase activity of samples should be between 2-35 nmol/min/ml. If it falls beyond this range, samples should be diluted with Ready Sample Buffer to bring enzymatic activity to this level.*
- It is advised to use multichannel pipettes to reduce dispensing time between wells.
- Dilute samples with Ready R2 buffer if absorbance is high in the sample wells (over 1.2).
- SDS at any concentration interferes in the assay.

Assay Protocol

1. Shake all samples for homogenation.
2. **Formaldehyde standard wells:** Add 100 µl *ready R1 reagent*, 30 µl Methanol (**R4**) and 20 µl standards (tubes A-G) to related wells.
3. **Sample wells:** Add 100 µl *ready R1 reagent*, 30 µl Methanol (**R4**) and 20 µl samples to related wells.
4. Initiate the reaction by adding 20 µl of diluted H_2O_2 to all of wells being used.
5. Cover the plate and incubate at room temperature for 20 mins on a shaker.
6. Add 30 µl of Stopper Reagent (**R5**) to each well for termination of the reaction and then add 30 µl Chromogenic Reagent (**R6**) to each well.
7. Cover the plate and shake for 10 mins at room temperature.
8. Add 10 µl of Oxidizer Reagent (**R7**) to each well.
9. Cover the plate and shake for 5 min at room temperature.
10. Read the absorbance at 540 nm using a plate reader.

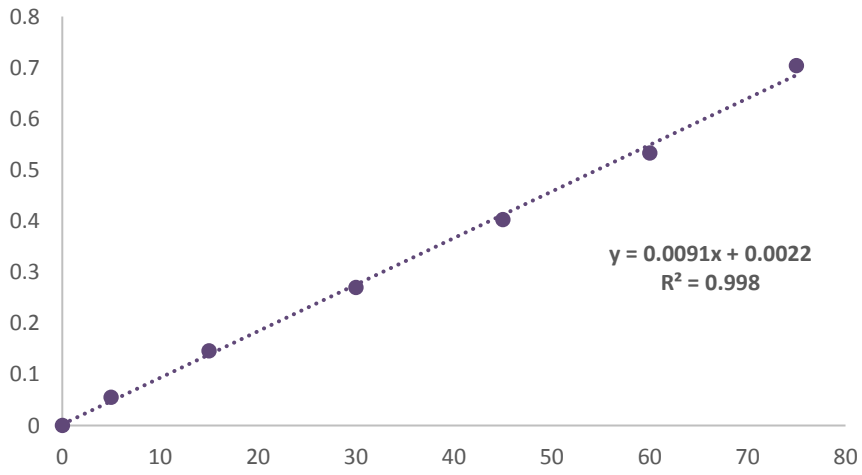
Calculations

1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of the standard A (0 µM) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Plot the corrected absorbance values of each standard as a function of final formaldehyde (µM) concentration.
4. Calculate the formaldehyde concentration of each Sample using the equation obtained from the standard curve. A typical standard curve is shown below (Fig. 2).

$$\text{Formaldehyde (µM)} = \frac{(Sample\ average\ absorbance) - (\text{y-intercept})}{Slope} \times \frac{0.17\ ml}{0.02\ \text{ml}}$$

5. Calculate the CAT activity using the following equation; one unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per min at 25°C.

$$\text{CAT Activity} = \frac{(\text{µM of } Sample)}{20\ min} \times \text{sample dilution} = \text{nmol/min/ml}$$



Formaldehyde Final Conc.	Corrected Absorbance
0	0
5	0.055
15	0.146
30	0.270
45	0.403
60	0.533
75	0.704

Fig. 2: Typical standard curve