



Catalase Assay Kit

Item No. 707002

www.caymanchem.com

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
707010	Catalase Assay Buffer (10X)	1 vial/5 ml	2 vials/5 ml
707012	Catalase Sample Buffer (10X)	1 vial/10 ml	2 vials/10 ml
707014	Catalase Formaldehyde Standard	1 vial/100 µl	1 vial/100 µl
707013	Catalase (Control)	1 vial/lyophilized	2 vials/lyophilized
707015	Catalase Potassium Hydroxide	1 vial/4 ml	5 vials/4 ml
707011	Catalase Hydrogen Peroxide	1 vial/1 ml	1 vial/1 ml
707017	Catalase Purpald (Chromogen)	1 vial/4 ml	5 vials/4 ml
707018	Catalase Potassium Periodate	1 vial/1.5 ml	5 vials/1.5 ml
400010	96-Well Clear Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 cover	5 covers

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

It is recommended to take appropriate precautions when using the kit reagents (*i.e.*, lab coat, gloves, eye goggles, etc.) as some of them can be harmful.

Catalase Hydrogen Peroxide is corrosive and is harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. Keep away from combustible materials.*

Catalase Formaldehyde Standard is carcinogenic. It is toxic if inhaled, ingested, or if in contact with skin. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. Keep away from combustible materials.*

Catalase Potassium Hydroxide is corrosive and is harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. Keep away from combustible materials.*

Catalase Purpald (Chromogen) is an irritant. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.*

Catalase Potassium Periodate is an oxidizer and an irritant. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.*

Hydrochloric acid is corrosive and is harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.*

*Before use the user must review the complete Material Safety Data Sheet.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

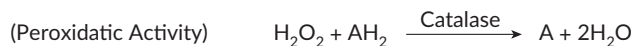
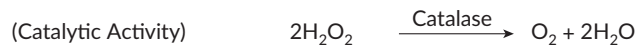
This kit will perform as specified if stored at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with a 540 nm filter.
2. An adjustable pipettor and a repeating pipettor.
3. A source of pure water. Glass distilled water or HPLC-grade water is acceptable.
4. A 5 ml vial of methanol can be purchased from Cayman (Item No. 707016).

Background

Catalase (EC 1.11.1.6; $2\text{H}_2\text{O}_2$ oxidoreductase) is an ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase (CAT) is involved in the detoxification of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), which is a toxic product of both normal aerobic metabolism and pathogenic ROS production. This enzyme catalyzes the conversion of two molecules of H_2O_2 to molecular oxygen and two molecules of water (catalytic activity). CAT also demonstrates peroxidatic activity, in which low molecular weight alcohols can serve as electron donors. While aliphatic alcohols serve as specific substrates for CAT, other enzymes with peroxidatic activity do not utilize these substrates.



In humans, the highest levels of CAT are found in liver, kidney, and erythrocytes, where it is believed to account for the majority of H_2O_2 decomposition.

About This Assay

Cayman's Catalase Assay Kit utilizes the peroxidatic function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2 . The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen.^{1,2} Purpald specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colorless to a purple color.^{1,2} The assay can be used to measure CAT activity in plasma, serum, erythrocyte lysates, tissue homogenates, and cell lysates.

PRE-ASSAY PREPARATION

Reagent Preparation

NOTE: Methanol is no longer supplied in this kit. It can be purchased separately under Item No. 707016 or you can supply your own.

1. Catalase Assay Buffer (10X) – (Item No. 707010)

Each vial contains 5 ml of Assay Buffer. Dilute 2 ml of Catalase Assay Buffer concentrate with 18 ml of HPLC-grade water. This final Catalase Assay Buffer (1X) (100 mM potassium phosphate, pH 7.0) should be used in the assay. When stored at 4°C, it is stable for at least two months. Prepare the additional vial as needed.

2. Catalase Sample Buffer (10X) – (Item No. 707012)

Each vial contains 10 ml of Sample Buffer. Dilute 5 ml of Catalase Sample Buffer concentrate with 45 ml of HPLC-grade water. This final Catalase Sample Buffer (1X) (25 mM potassium phosphate, pH 7.5, containing 1 mM EDTA and 0.1% BSA) should be used to dilute the formaldehyde standards, Catalase (Control), and CAT samples prior to assaying. When stored at 4°C, the Catalase Sample Buffer (1X) is stable for at least two months. Prepare the additional vial as needed.

3. Catalase Formaldehyde Standard - (Item No. 707014)

The vial contains 4.25 M formaldehyde. The reagent is ready to use as supplied.

4. Catalase (Control) – (Item No. 707013)

Each vial contains a lyophilized powder of bovine liver CAT and is used as a positive control. Reconstitute the Catalase (Control) by adding 2 ml of Catalase Sample Buffer (1X) to the vial and vortex well. Take 100 µl of the reconstituted enzyme and dilute with 1.9 ml of Catalase Sample Buffer (1X). A 20 µl aliquot of this diluted enzyme per well causes an absorbance between 0.25-0.45 OD before subtracting the background. The diluted enzyme is stable for 30 minutes. The reconstituted Catalase (Control) is stable for one month at -20°C.

5. Catalase Potassium Hydroxide – (Item No. 707015)

Each vial contains 4 ml of 10 M potassium hydroxide (KOH). The reagent is ready to use as supplied.

6. Catalase Hydrogen Peroxide – (Item No. 707011)

The vial contains an 8.82 M solution of H₂O₂. Dilute 40 µl of Catalase Hydrogen Peroxide with 9.96 ml of HPLC-grade water. The diluted Hydrogen Peroxide solution is stable for two hours.

7. Catalase Purpald (Chromogen) – (Item No. 707017)

Each vial contains 4 ml of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) in 0.5 M hydrochloric acid. The reagent is ready to use as supplied.

8. Catalase Potassium Periodate – (Item No. 707018)

Each vial contains 1.5 ml of potassium periodate in 0.5 M potassium hydroxide. The reagent is ready to use as supplied.

Sample Preparation

Overheating can inactivate catalase. The enzyme should be kept cold during sample preparation and assaying. In general, catalase is very unstable at high dilution. It is recommended to store samples concentrated and assay within 30 minutes after dilution.

Tissue Homogenate

1. Prior to dissection, either perfuse tissue or rinse tissue with a phosphate buffered saline (PBS) solution, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue on ice in 5-10 ml of cold buffer (*i.e.*, 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA) per gram tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

Cell Lysate

1. Collect cells by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman.
2. Homogenize or sonicate the cell pellet on ice in 1-2 ml of cold buffer (*i.e.*, 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

Plasma and Erythrocyte Lysate

1. Collect blood using an anticoagulant such as heparin, citrate, or EDTA.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80°C. The plasma sample will be stable for at least one month.
3. Remove the white buffy layer (leukocytes) and discard.
4. Lyse the erythrocytes (red blood cells) in four times its volume of ice-cold HPLC-grade water.
5. Centrifuge at 10,000 x g for 15 minutes at 4°C.
6. Collect the supernatant (erythrocyte lysate) for assaying and store on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for at least one month.

Serum

1. Collect blood without using an anticoagulant. Allow blood to clot for 30 minutes at 25°C.
2. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for at least one month.

Tissue Homogenization using the Precellys 24 Homogenizer

- Prior to dissection, either perfuse or rinse tissue with phosphate buffered saline (PBS), pH 7.4, to remove any red blood cells and clots.
- Freeze organs immediately upon collection and then store at -80°C. Snap-freezing of tissues in liquid nitrogen is preferred.
- Add cold 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA.
- Homogenize the tissue sample using the Precellys 24 according to appropriate settings.
- Spin the tissue homogenates at 10,000 x g for 15 minutes at 4°C.
- Collect supernatant and assay samples according to the kit booklet protocol. Samples may need to be diluted appropriately for assay and should be normalized using a protein assay.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. We suggest that there be at least two wells designated as positive controls. A typical layout of formaldehyde standards and samples to be measured in duplicate is shown in Figure 1. We suggest you record the contents of each well on the template sheet provided on page 22.

	1	2	3	4	5	6	7	8	9	10	11	12
A	(A)	(A)	(S1)	(S1)	(S9)	(S9)	(S17)	(S17)	(S25)	(S25)	(S33)	(S33)
B	(B)	(B)	(S2)	(S2)	(S10)	(S10)	(S18)	(S18)	(S26)	(S26)	(S34)	(S34)
C	(C)	(C)	(S3)	(S3)	(S11)	(S11)	(S19)	(S19)	(S27)	(S27)	(S35)	(S35)
D	(D)	(D)	(S4)	(S4)	(S12)	(S12)	(S20)	(S20)	(S28)	(S28)	(S36)	(S36)
E	(E)	(E)	(S5)	(S5)	(S13)	(S13)	(S21)	(S21)	(S29)	(S29)	(S37)	(S37)
F	(F)	(F)	(S6)	(S6)	(S14)	(S14)	(S22)	(S22)	(S30)	(S30)	(S38)	(S38)
G	(G)	(G)	(S7)	(S7)	(S15)	(S15)	(S23)	(S23)	(S31)	(S31)	(S39)	(S39)
H	(+)	(+)	(S8)	(S8)	(S16)	(S16)	(S24)	(S24)	(S32)	(S32)	(S40)	(S40)

A-G = Standards
+ = Positive controls
S1-S40 = Sample wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 240 μ l in all the wells.
- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- If the expected CAT activity of the sample is not known or if it is expected to be beyond the range of the standard curve, it is prudent to assay the sample at several dilutions.
- It is recommended that the samples and formaldehyde standards be assayed at least in duplicate.
- Use the Catalase Sample Buffer (1X) in the assay.
- Monitor the absorbance at 540 nm using a plate reader.

Standard Preparation

Preparation of the Formaldehyde Standards - Dilute 10 μ l of Catalase Formaldehyde Standard (Item No. 707014) with 9.99 ml of Catalase Sample Buffer (1X) to obtain a 4.25 mM formaldehyde stock solution. Take seven clean glass test tubes and mark them A-G. Add the amount of formaldehyde stock and Catalase Sample Buffer (1X) to each tube as described in Table 1 (below).

Tube	Formaldehyde (μ l)	Catalase Sample Buffer (1X) (μ l)	Final Concentration (μ M formaldehyde)*
A	0	1,000	0
B	10	990	5
C	30	970	15
D	60	940	30
E	90	910	45
F	120	880	60
G	150	850	75

Table 1

*Final formaldehyde concentration in the 170 μ l reaction.

Performing the Assay

1. **Formaldehyde Standard Wells** - Add 100 μl of Catalase Assay Buffer (1X), 30 μl of methanol, and 20 μl of standard (tubes A-G) per well in the designated wells on the plate (see **Sample plate format**, Figure 1, page 13).
2. **Positive Control Wells (bovine liver CAT)** - Add 100 μl of Catalase Assay Buffer (1X), 30 μl of methanol, and 20 μl of diluted Catalase (Control) to two wells.
3. **Sample Wells** - Add 100 μl of Catalase Assay Buffer (1X), 30 μl of methanol, and 20 μl of sample to two wells. When necessary, samples should be diluted with Catalase Sample Buffer (1X) or concentrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 100,000.
4. Initiate the reactions by adding 20 μl of diluted Hydrogen Peroxide to all the wells being used. Make sure to note the precise time the reaction is initiated and add the diluted Hydrogen Peroxide as quickly as possible.
5. Cover the plate with the plate cover and incubate on a shaker for 20 minutes at room temperature.
6. Add 30 μl of Potassium Hydroxide to each well to terminate the reaction and then add 30 μl of Catalase Purpald (Chromogen) (Item No. 707017) to each well.
7. Cover the plate with the plate cover and incubate for 10 minutes at room temperature on the shaker.
8. Add 10 μl of Catalase Potassium Periodate (Item No. 707018) to each well. Cover with plate cover and incubate five minutes at room temperature on a shaker.
9. Read the absorbance at 540 nm using a plate reader.

ANALYSIS

Calculations

Determination of the Reaction Rate

1. Calculate the average absorbances of each standard and sample.
2. Subtract the average absorbance of standard A from itself and all other standards and samples.
3. Plot the corrected absorbance of standards (from step 2 above) as a function of final formaldehyde concentration (μM) from Table 1. See Figure 2 for a typical standard curve.

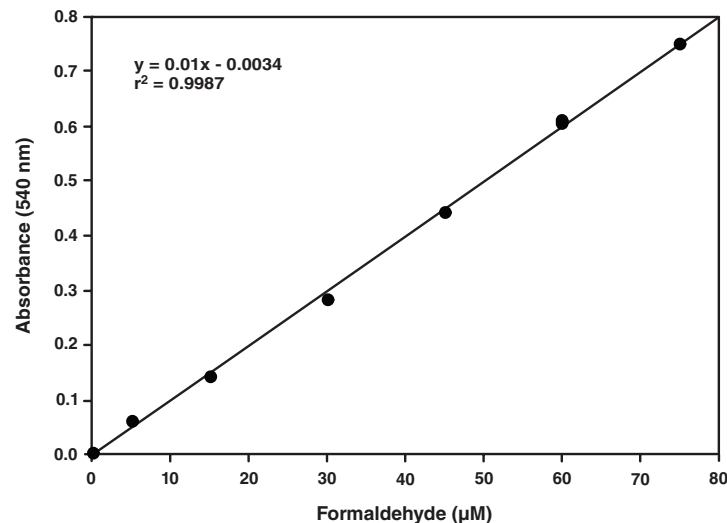


Figure 2. Formaldehyde standard curve

4. Calculate the formaldehyde concentration of the samples using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each sample.

$$\text{Formaldehyde } (\mu\text{M}) = \left[\frac{\text{Sample absorbance} - (\text{y-intercept})}{\text{Slope}} \right] \times \frac{0.17 \text{ ml}}{0.02 \text{ ml}}$$

5. Calculate the CAT activity of the sample 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 minute at 25°C.

$$\text{CAT Activity} = \frac{\mu\text{M of Sample}}{20 \text{ min.}} \times \text{Sample dilution} = \text{nmol/min/ml}$$

Performance Characteristics

Sensitivity:

The Lower Limit of Detection (LLOD) for this assay is 2 nmol/min/ml. The assay is linear up to 96 nmol/min/ml, unless limited by the accuracy of absorbance measurement.

Precision:

When a series of 45 CAT measurements were performed on the same day, the intra-assay coefficient of variation was 3.8%. When a series of 45 CAT measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 9.9%.

Linearity of the Assay

The dose-response relationship for purified CAT from bovine liver was linear from 5-80 ng of protein (see Figure 3, below). Tissue homogenates, cell lysates, plasma, serum, and erythrocyte lysates also exhibited a linear relationship between the amount of sample and CAT activity over a wide range.

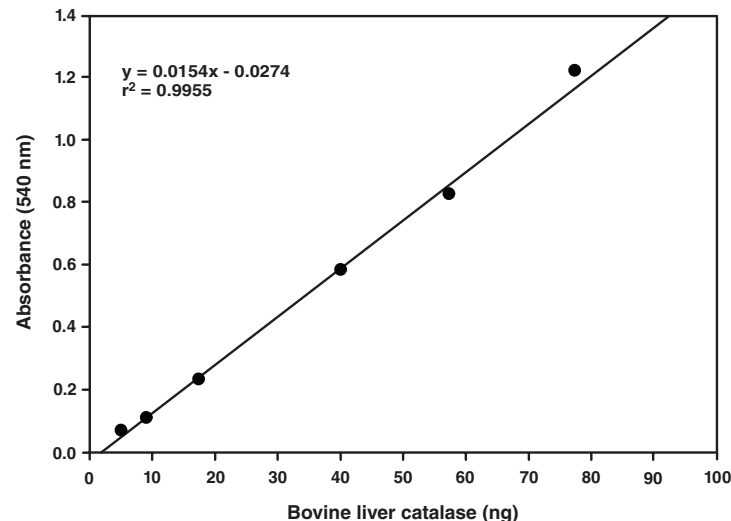


Figure 3. Absorbance versus bovine liver catalase (ng)

Interferences

The following reagents were tested for interference in the assay.

	Reagent	Will Interfere (Yes or No)
Detergents:	SDS (precipitates at pH 7.0)	Yes
	Triton X-100 ($\leq 1\%$)	No
	Polysorbate 20 ($\leq 1\%$)	No
	CHAPS ($\leq 1\%$)	No
Buffers:	Tris	No
	HEPES	No
	Phosphate	No
Protease Inhibitors/Chelators:	Antipain (≤ 0.1 mg/ml)	No
	PMSF (≤ 1 mM)	No
	Leupeptin (≤ 1 mg/ml)	No
	Trypsin (≤ 0.1 mg/ml)	No
	Chymostatin (≤ 1 mg/ml)	No
	EGTA (≤ 1 mM)	No
	EDTA (≤ 1 mM)	No
Others:	NADPH (≤ 2 μ M)	No
	Glycerol ($\leq 1\%$)	No
	BSA (≤ 1 mg/ml)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s) C. Poor timing between addition of the reagents into the well(s)	A. Be careful not to splash the contents of the wells B. Scan immediately after the last incubation. Carefully tap the side of the plate with your finger to remove bubbles. If the wells are covered with bubbles, re-scan the plate after centrifuging the plate at 1,000 x g for 1 minute without the plate cover or after popping the bubbles with a syringe needle C. Add reagents to the well(s) within less than one minute
No activity was detected in the sample	A. Catalase activity was too low B. Sample was too dilute	Concentrate the samples using an Amicon concentrator with a molecular weight cut-off of 100,000 and re-assay
Absorbance over 1.2 in the sample wells	Too much enzyme was added to well(s)	Dilute samples with diluted Sample Buffer and re-assay
Absorbance of standard A is >0.2	A. The methanol is contaminated B. Extended time between last incubation and the plate reading	A. Re-assay using methanol from a fresh container B. Read the plate immediately after the last incubation step

References

- Johansson, L.H. and Borg, L.A.H. *Anal. Biochem.* **174**, 331-336 (1988).
- Wheeler, C.R., Salzman, J.A., Elsayed, N.M., *et al. Anal. Biochem.* **184**, 193-199 (1990).

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NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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