

Catalase Assay Kit

Catalog No. 707002

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Item	Quantity
707010	Assay Buffer (10X)	1 vial
707012	Sample Buffer (10X)	1 vial
707014	Formaldehyde Standard	1 vial
707013	Catalase (control)	1 vial
707015	Potassium Hydroxide	1 vial
707011	Hydrogen Peroxide	1 vial
707017	Purpald (chromagen)	1 vial
707018	Potassium Periodate	1 vial
400014	96-Well Plate	1 plate
400012	96-Well Cover Sheets	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only; not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay. For research use only. Not for human or diagnostic use.

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.

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.*

Formaldehyde 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.*

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. Heat is generated when potassium hydroxide pellets are dissolved in water.*

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

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

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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

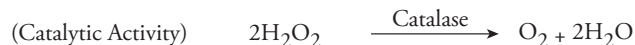
This kit will perform as specified if stored at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

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

Background

Catalase (EC 1.11.1.6; 2H₂O₂ oxidoreductase) is an ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase (CAT) is involved in the detoxification of hydrogen peroxide (H₂O₂), 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 H₂O₂ 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 catalase are found in liver, kidney, and erythrocytes, where it is believed to account for the majority of hydrogen peroxide 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 H₂O₂. 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 Catalog No. 707016 or you can supply your own.

1. Assay Buffer (10X) – (Catalog No. 707010)

Dilute 2 ml of Assay Buffer concentrate with 18 ml of HPLC-grade water. This final Assay Buffer (100 mM potassium phosphate, pH 7.0) should be used in the assay. When stored at 4°C, this diluted Assay Buffer is stable for at least two months.

2. Sample Buffer (10X) – (Catalog No. 707012)

Dilute 5 ml of Sample Buffer concentrate with 45 ml of HPLC-grade water. This final Sample Buffer (25 mM potassium phosphate, pH 7.5, containing 1 mM EDTA and 0.1% BSA) should be used to dilute the formaldehyde standards, CAT (positive control), and CAT samples prior to assaying. When stored at 4°C, this diluted Sample Buffer is stable for at least two months.

3. Formaldehyde Standard - (Catalog No. 707014)

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

4. Catalase (control) – (Catalog No. 707013)

This vial contains a lyophilized powder of bovine liver CAT and is used as a positive control. Reconstitute the CAT by adding 2 ml of Sample Buffer (dilute) to the vial and vortex well. Take 100 µl of the reconstituted enzyme and dilute with 1.9 ml of Sample Buffer (dilute). A 20 µl aliquot of this diluted enzyme per well causes an absorbance of approximately 0.29 after subtracting the background absorbance. The diluted enzyme is stable for 30 minutes. The reconstituted CAT is stable for one month at -20°C.

5. Potassium hydroxide – (Catalog No. 707015)

The vial contains potassium hydroxide (KOH) pellets. Place the vial on ice, add 4 ml of cold HPLC-grade water, and vortex to yield a 10 M solution. *CAUTION: Heat is generated when potassium hydroxide pellets are dissolved in water. The KOH solution is stable for at least three months if stored at 4°C.*

6. Hydrogen peroxide – (Catalog No. 707011)

This vial contains an 8.82 M solution of hydrogen peroxide. Dilute 40 µl of hydrogen peroxide with 9.96 ml of HPLC-grade water. The dilute hydrogen peroxide solution is stable for two hours.

7. Purpald (chromogen) – (Catalog No. 707017)

This vial contains a solution 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. Potassium Periodate – (Catalog No. 707018)

This vial contains a solution 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.

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 23.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. A	Std. A	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
B	Std. B	Std. B	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
C	Std. C	Std. C	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
D	Std. D	Std. D	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
E	Std. E	Std. E	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
F	Std. F	Std. F	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
G	Std. G	Std. G	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39
H	CAT control	CAT control	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32	Sample 40	Sample 40

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 Assay Buffer (dilute) in the assay.
- Monitor the absorbance at 540 nm using a plate reader.

Standard Preparation

1. Preparation of the Formaldehyde Standards - Dilute 10 μl of formaldehyde standard (vial #3) with 9.99 ml of Sample Buffer (dilute) 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 Sample Buffer (dilute) to each tube as described in Table 1 (below).

Tube	Formaldehyde (μl)	Sample Buffer (μl)	Final Concentration (μM 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 μl reaction.

Performing the Assay

1. **Formaldehyde Standard Wells** - Add 100 μl of Assay Buffer (dilute), 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 12).
2. **Positive Control Wells (bovine liver CAT)** - Add 100 μl of Assay Buffer (dilute), 30 μl of methanol, and 20 μl of diluted CAT (control) to two wells.
3. **Sample Wells** - Add 100 μl of Assay Buffer (dilute), 30 μl of methanol, and 20 μl of sample to two wells. To obtain reproducible results, the amount of CAT added to the well should result in an activity between 0.25-4 nmol/min/ml. When necessary, samples should be diluted with Sample Buffer (dilute) or concentrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 100,000 to bring the enzymatic activity to this level.
4. Initiate the reactions by adding 20 μl of hydrogen peroxide (dilute) to all the wells being used. Make sure to note the precise time the reaction is initiated and add the 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 Purpald (chromogen) 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 potassium periodate 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.

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.

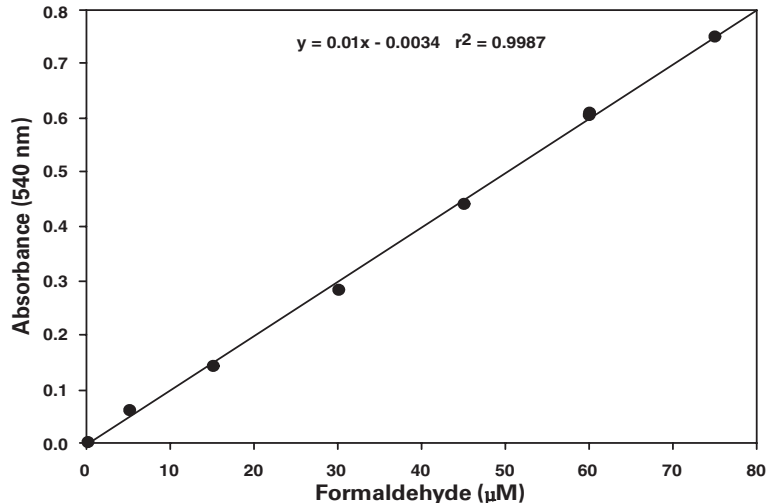


Figure 2. Formaldehyde standard curve

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 Catalase 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 dynamic range of the assay is limited only by the accuracy of the absorbance measurement. Most plate readers are linear to an absorbance of 1.2. Samples containing CAT activity between 2-34 nmol/min/ml can be assayed without further dilution or concentration.

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 catalase 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 catalase activity over a wide range.

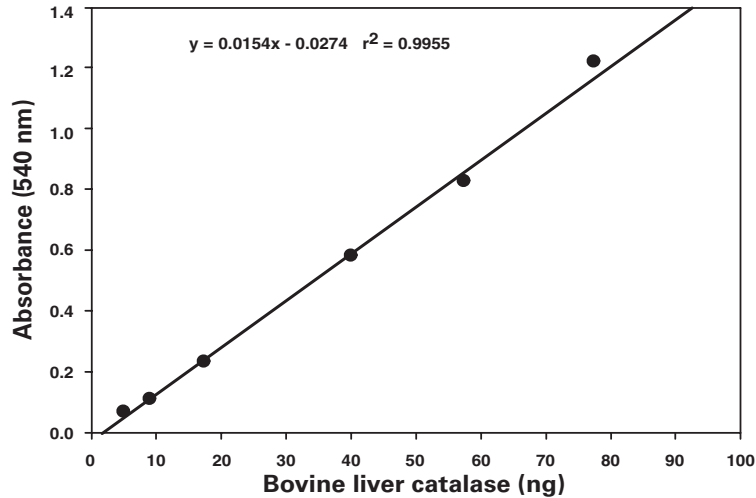


Figure 3. Absorbance versus bovine liver catalase (ng)

RESOURCES

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
	Tween 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)	A. Carefully tap the side of the plate with your finger to remove bubbles B. Be careful not to splash the contents of the wells
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 sample buffer (dilute) and re-assay.
Absorbance of standard A is >0.2	The methanol is contaminated	Re-assay using methanol from a fresh container

References

1. Johansson, L.H. and Borg, L.A.H. A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal. Biochem.* **174**, 331-336 (1988).
2. Wheeler, C.R., Salzman, J.A., Elsayed, N.M., *et al.* Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. *Anal. Biochem.* **184**, 193-199 (1990).

Related Products

Aconitase Assay Kit - Cat. No. 705502
Antioxidant Assay Kit - Cat. No. 709001
Glutathione Assay Kit - Cat. No. 703002
Glutathione Peroxide Assay Kit - Cat. No. 703102
Glutathione Reductase Assay Kit - Cat. No. 703202
Glutathione S-Transferase Assay Kit - Cat. No. 703302
Hydrogen Peroxide (urinary) Assay Kit - Cat. No. 706011
8-Isoprostane EIA Kit - Cat. No. 516351
Lipid Hydroperoxide Assay Kit - Cat. No. 705002
Myeloperoxidase Chlorination Assay Kit - Cat. No. 10006438
Myeloperoxidase Inhibitor Screening Assay Kit - Cat. No. 700170
Myeloperoxidase Peroxidation Assay Kit - Cat. No. 700160
Protein Carbonyl Assay Kit - Cat. No. 10005020
Superoxide Dismutase Assay Kit - Cat. No. 706002
TBARS Assay Kit - Cat. No. 10009055
Thioredoxin Reductase Assay Kit - Cat. No. 10007892
Xanthine Oxidase Assay Kit - Cat. No. 10010895

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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	A	B	C	D	E	F	G	H

NOTES

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