Catalase Assay Kit
(without Hydrogen Peroxide)

Item No. 700910

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Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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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
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	High-Binding 96-Well Solid 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 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
Fax:	734-971-3641
Email:	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 capable of reading absorbance at 540 nm.
2. An adjustable pipettor and a repeating or multichannel pipettor.
3. A source of pure water. Glass distilled water or HPLC-grade water is acceptable.
4. Methanol (a 5 ml vial of methanol can be purchased from Cayman (Item No. 707016)).
5. A supply of 8.82 M (30%) Hydrogen Peroxide. A 1 ml vial of Hydrogen Peroxide can be purchased separately from Cayman (Item No. 707011).

INTRODUCTION

Background

Catalase (EC 1.11.1.6; $2H_2O_2$ 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.

(Catalytic Activity) $2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O$

(Peroxidatic Activity) $H_2O_2 + AH_2 \xrightarrow{\text{Catalase}} A + 2H_2O$

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

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 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. 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 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, Catalase (Control), and CAT samples prior to assaying. When stored at 4°C, this diluted Sample Buffer 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 diluted Sample Buffer to the vial and vortex well. Take 100 µl of the reconstituted enzyme and dilute with 1.9 ml of diluted Sample Buffer. A 20 µl aliquot of this diluted enzyme per well produces an absorbance of approximately 0.29 after subtracting the background absorbance. 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 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.

7. **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.

8. **Hydrogen Peroxide Solution – (not supplied in kit)**

   Obtain a source of 8.82 M (30%) solution of hydrogen peroxide. Dilute 40 µl of the hydrogen peroxide with 9.96 ml of HPLC-grade water. The diluted Hydrogen Peroxide Solution is stable for two hours.

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. Carefully pipette off the top yellow plasma layer without disturbing the white buffy layer and transfer to a clear tube. 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. Carefully pipette off the top yellow serum layer without disturbing the white buffy layer layer and transfer to a clear tube. 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.
- Centrifuge at 10,000 x g for 15 minutes at 4°C.
- Collect the 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 µl 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.
- It is recommended that the samples and formaldehyde standards be assayed at least in duplicate, and that samples be analyzed at more than one dilution.
- Use the diluted Assay Buffer 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 Catalase Formaldehyde Standard (Item No. 707014) with 9.99 ml of diluted Sample Buffer 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 diluted Sample Buffer 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 diluted Assay Buffer, 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 diluted Assay Buffer, 30 µl of methanol, and 20 µl of diluted Catalase (Control) to two wells.
3. **Sample Wells -** Add 100 µl of diluted Assay Buffer, 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 2-35 nmol/min/ml. When necessary, samples can be adjusted to this activity level by either diluting with diluted Sample Buffer, or concentrated using a filter with a molecular weight cut-off of 100,000 kDa.
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 diluted 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 absorbance 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.

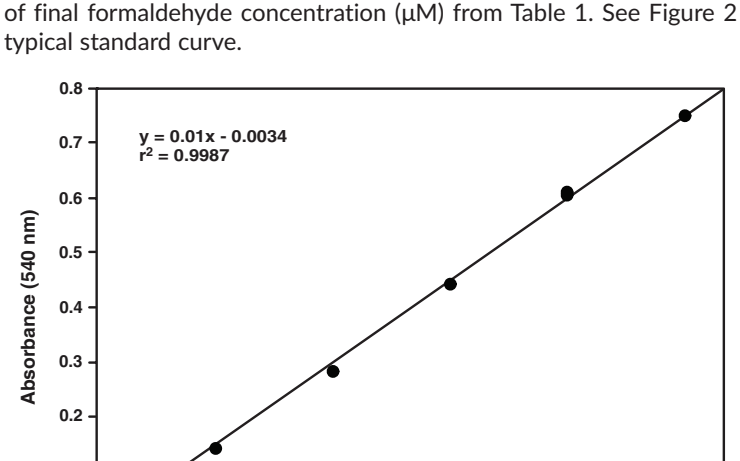


Figure 2. Formaldehyde standard curve

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.

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

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.

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

Performance Characteristics

Assay Range:

Samples containing CAT activity between 2-35 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 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.

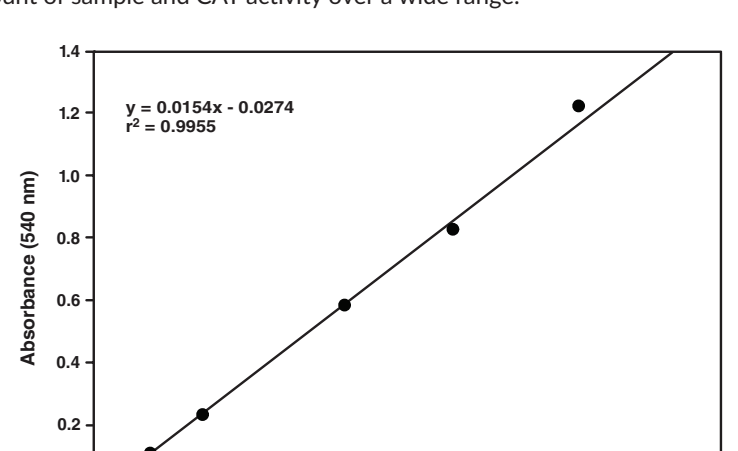


Figure 3. Dose-response relationship for bovine CAT

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

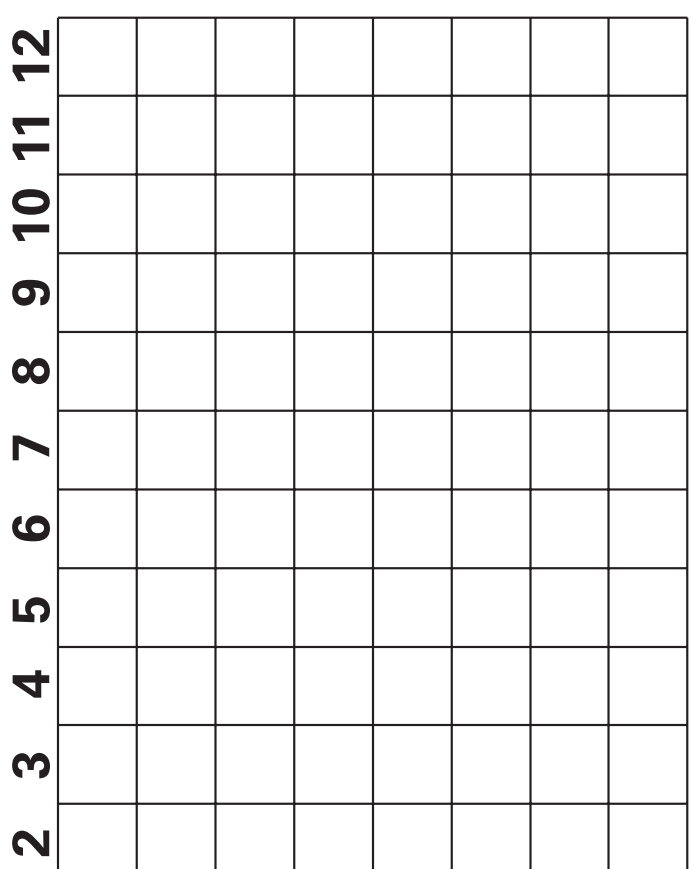
	Reagent	Will Interfere (Yes or No)
Detergents:	SDS (precipitates at pH 7.0)	Yes
	Triton X-100 (≤1%)	No
	Polysorbate 20 (≤1%)	No
	CHAPS (≤1%)	No
Buffers:	Tris	No
	HEPES	No
	Phosphate	No
Protease Inhibitors/ Chelators:	Antipain (≤0.1 mg/ml)	No
	PMSF (≤1 mM)	No
	Leupeptin (≤1 mg/ml)	No
	Trypsin (≤0.1 mg/ml)	No
	Chymostatin (≤1 mg/ml)	No
	EGTA (≤1 mM)	No
	EDTA (≤1 mM)	No
Others:	NADPH (≤2 µM)	No
	Glycerol (≤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. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No activity was detected in the sample	A. Catalase activity was too low B. Sample was too dilute	Concentrate the samples using a molecular weight cut-off filter 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	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).



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.