Catalase Assay Kit
(without Hydrogen Peroxide)

Item No. 700910

www.caymanchem.com
Customer Service 800.364.9897
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**GENERAL INFORMATION**

**Materials Supplied**

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>96 wells Quantity/Size</th>
<th>480 wells Quantity/Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>707010</td>
<td>Catalase Assay Buffer (10X)</td>
<td>1 vial/5 ml</td>
<td>2 vials/5 ml</td>
</tr>
<tr>
<td>707012</td>
<td>Catalase Sample Buffer (10X)</td>
<td>1 vial/10 ml</td>
<td>2 vials/10 ml</td>
</tr>
<tr>
<td>707014</td>
<td>Catalase Formaldehyde Standard</td>
<td>1 vial/100 μl</td>
<td>1 vial/100 μl</td>
</tr>
<tr>
<td>707013</td>
<td>Catalase (Control)</td>
<td>1 vial/lyophilized</td>
<td>2 vials/lyophilized</td>
</tr>
<tr>
<td>707015</td>
<td>Catalase Potassium Hydroxide</td>
<td>1 vial/4 ml</td>
<td>5 vials/4 ml</td>
</tr>
<tr>
<td>707017</td>
<td>Catalase Purpald (Chromogen)</td>
<td>1 vial/4 ml</td>
<td>5 vials/4 ml</td>
</tr>
<tr>
<td>707018</td>
<td>Catalase Potassium Periodate</td>
<td>1 vial/1.5 ml</td>
<td>5 vials/1.5 ml</td>
</tr>
<tr>
<td>400010</td>
<td>High-Binding 96-Well Solid Plate</td>
<td>1 plate</td>
<td>5 plates</td>
</tr>
<tr>
<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>1 cover</td>
<td>5 covers</td>
</tr>
</tbody>
</table>

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; 2H₂O₂ oxido-reductase) 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.

\[
\text{Catalytic Activity: } 2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} \text{O}_2 + 2\text{H}_2\text{O}
\]

\[
\text{Peroxidatic Activity: } \text{H}_2\text{O}_2 + \text{AH}_2 \xrightarrow{\text{Catalase}} \text{A} + 2\text{H}_2\text{O}
\]

In humans, the highest levels of CAT are found in liver, kidney, and erythrocytes, where it is believed to account for the majority of H₂O₂ 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.\(^1\) Purpald specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colorless to a purple color.\(^1\) 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.
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.

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.
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 S2 S3 S4 S5 S6 S7 S8 S9 S10
B B B S11 S12 S13 S14 S15 S16 S17 S18 S19 S20
C C C S21 S22 S23 S24 S25 S26 S27 S28 S29 S30
D D D S31 S32 S33 S34 S35 S36 S37 S38 S39 S40
E E E S41 S42 S43 S44 S45 S46 S47 S48 S49 S50
F F F S51 S52 S53 S54 S55 S56 S57 S58 S59 S60
G G G S61 S62 S63 S64 S65 S66 S67 S68 S69 S70
H + + + S71 S72 S73 S74 S75 S76 S77 S78 S79 S80
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 pipetted 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).

<table>
<thead>
<tr>
<th>Tube</th>
<th>Formaldehyde (μl)</th>
<th>Sample Buffer (μl)</th>
<th>Final Concentration (μM formaldehyde)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>1,000</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>990</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>970</td>
<td>15</td>
</tr>
<tr>
<td>D</td>
<td>60</td>
<td>940</td>
<td>30</td>
</tr>
<tr>
<td>E</td>
<td>90</td>
<td>910</td>
<td>45</td>
</tr>
<tr>
<td>F</td>
<td>120</td>
<td>880</td>
<td>60</td>
</tr>
<tr>
<td>G</td>
<td>150</td>
<td>850</td>
<td>75</td>
</tr>
</tbody>
</table>

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.
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.

\[
y = 0.01x - 0.0034 \\
r^2 = 0.9987
\]

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.

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

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.

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

Figure 2. Formaldehyde standard curve
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

\[ y = 0.0154x - 0.0274 \]
\[ r^2 = 0.9955 \]
## Interferences

The following reagents were tested for interference in the assay.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Will Interfere (Yes or No)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Detergents:</strong></td>
<td></td>
</tr>
<tr>
<td>SDS (precipitates at pH 7.0)</td>
<td>Yes</td>
</tr>
<tr>
<td>Triton X-100 (≤1%)</td>
<td>No</td>
</tr>
<tr>
<td>Polysorbate 20 (≤1%)</td>
<td>No</td>
</tr>
<tr>
<td>CHAPS (≤1%)</td>
<td>No</td>
</tr>
<tr>
<td><strong>Buffers:</strong></td>
<td></td>
</tr>
<tr>
<td>Tris</td>
<td>No</td>
</tr>
<tr>
<td>HEPES</td>
<td>No</td>
</tr>
<tr>
<td>Phosphate</td>
<td>No</td>
</tr>
<tr>
<td><strong>Protease Inhibitors/Chelators:</strong></td>
<td></td>
</tr>
<tr>
<td>Antipain (≤0.1 mg/ml)</td>
<td>No</td>
</tr>
<tr>
<td>PMSF (≤1 mM)</td>
<td>No</td>
</tr>
<tr>
<td>Leupeptin (≤1 mg/ml)</td>
<td>No</td>
</tr>
<tr>
<td>Trypsin (≤0.1 mg/ml)</td>
<td>No</td>
</tr>
<tr>
<td>Chymostatin (≤1 mg/ml)</td>
<td>No</td>
</tr>
<tr>
<td>EGTA (≤1 mM)</td>
<td>No</td>
</tr>
<tr>
<td>EDTA (≤1 mM)</td>
<td>No</td>
</tr>
<tr>
<td><strong>Others:</strong></td>
<td></td>
</tr>
<tr>
<td>NADPH (≤2 μM)</td>
<td>No</td>
</tr>
<tr>
<td>Glycerol (≤1%)</td>
<td>No</td>
</tr>
<tr>
<td>BSA (≤1 mg/ml)</td>
<td>No</td>
</tr>
</tbody>
</table>

## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erratic values; dispersion of duplicates/triplicates</td>
<td>A. Poor pipetting/technique</td>
<td>A. Be careful not to splash the contents of the wells</td>
</tr>
<tr>
<td></td>
<td>B. Bubble in the well(s)</td>
<td>B. Carefully tap the side of the plate with your finger to remove bubbles</td>
</tr>
<tr>
<td>No activity was detected in the sample</td>
<td>A. Catalase activity was too low</td>
<td>Concentrate the samples using a molecular weight cut-off filter of 100,000 and re-assay</td>
</tr>
<tr>
<td></td>
<td>B. Sample was too dilute</td>
<td>Dilute samples with diluted Sample Buffer and re-assay</td>
</tr>
<tr>
<td>Absorbance over 1.2 in the sample wells</td>
<td>Too much enzyme was added to well(s)</td>
<td>Re-assay using methanol from a fresh container</td>
</tr>
<tr>
<td>Absorbance of standard A is &gt;0.2</td>
<td>The methanol is contaminated</td>
<td>Re-assay using methanol from a fresh container</td>
</tr>
</tbody>
</table>

## References


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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