Technical Bulletin

Catalase Assay Kit

Catalog Number MAK381

Product Description

Catalase is a ubiquitous antioxidant enzyme that is present in nearly all living organisms. It functions to catalyze the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen.

The Catalase Assay Kit provides a highly sensitive, simple, direct, and high throughput assay for measuring catalase activity in biological samples. In the assay, catalase first reacts with H_2O_2 to produce water and oxygen. The unconverted H_2O_2 subsequently reacts with the probe to produce a chromogenic product which can be measured colorimetrically at 570 nm or fluorometrically at λ_{Ex} = 535 nm/λ_{Em} = 587 nm. Catalase activity is inversely proportional to the signal. The kit can detect 1 microunit (μU) or less of catalase activity in samples.

The kit is suitable for the determination of catalase

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Catalase Assay Buffer 25 mL
  Catalog Number MAK381A
- Probe (in DMSO) 200 μL
  Catalog Number MAK381B
- HRP (lyophilized) 1 vial
  Catalog Number MAK381C
- H_2O_2 (0.88 M) 25 μL
  Catalog Number MAK381D
- Stop Solution 1 mL
  Catalog Number MAK381E
- Catalase Positive Control 2 μL
  Catalog Number MAK381F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well flat-bottom plate. It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Fluorescence or spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2-8 °C protected from light.

Preparation Instructions

Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Catalase Assay Buffer: Warm the assay buffer to room temperature before use. Chill a small amount of Catalase Assay Buffer for use with Sample and Positive Control Preparation.

Probe (in DMSO): Briefly warm to completely melt the DMSO solution. Store at 2-8 °C, protected from light. Once opened, use within two months.

HRP: Reconstitute vial with 220 µL of Catalase Assay Buffer. Store at 2-8 °C. Use within two months.

Catalase Positive Control Solution: Add 500 µL of Catalase Assay Buffer to Catalase Positive Control. Aliquot and store at -20 °C. Diluted Catalase Positive Control Solution is stable for 2-3 days at 2-8 °C and for 2 months at -20 °C.

Sample and Positive Control Preparation

Note: Reducing agents in samples interfere with the assay. Keep DTT or β-Mercapto-ethanol in the sample below 5 µM.

1. Homogenize 0.1 gram tissues, or 10^6 cells, or 0.2 ml Erythrocytes on ice in 0.2 ml cold Catalase Assay Buffer. Centrifuge at 10,000 × g for 15 minutes at 2-8 °C.

   Collect the supernatant for assay and keep on ice.

2. Liquid samples can be tested directly. If samples will not be assayed immediately, store at -80 °C.

3. Add 2-78 µL of sample or 1-5 µL Catalase Positive Control Solution into each well and adjust volume to a final volume of 78 µL with Catalase Assay Buffer.

4. Prepare Sample High Control (HC) with the same amount of sample in separate wells and adjust volume to 78 µL with Catalase Assay Buffer. Add 10 µL of Stop Solution into the Sample HC, mix and incubate at 25 °C for 5 minutes to completely inhibit the catalase activity in samples as High Control.

5. For unknown samples, it is recommended to test several doses of sample to ensure the readings are within the linear range.

Procedure

Colorimetric Standard Curve Preparation

Note: Diluted H_2O_2 is unstable. Prepare a fresh dilution for each set of assays.

1. Prepare a 20 mM solution of H_2O_2 by diluting 5 µL of H_2O_2 (0.88 M) with 215 µL of purified water. Mix well.

2. Prepare a 1 mM H_2O_2 solution by diluting 50 µL of the 20 mM H_2O_2 solution with 0.95 mL of purified water. Mix well.

3. Prepare H_2O_2 Standards according to Table 1. Mix well.

Table 1.

Preparation of H_2O_2 Standards for Colorimetric assay

Well	1 mM H_2O_2 Standard	Catalase Assay Buffer	Stop Solution	H_2O_2 (nmol/well)
1	0 µL	90 µL	10 µL	0
2	2 µL	88 µL	10 µL	2
3	4 µL	86 µL	10 µL	4
4	6 µL	84 µL	10 µL	6
5	8 µL	82 µL	10 µL	8
6	10 µL	80 µL	10 µL	10

Fluorometric Standard Curve Preparation

Note: Diluted H_2O_2 is unstable. Prepare a fresh dilution for each set of assays.

1. Prepare a 1 mM H_2O_2 solution as per Steps 1 and 2 of the Colorimetric Standard Curve Preparation section.
2. Prepare a 0.1 mM H_2O_2 solution in purified water by diluting the 1 mM H_2O_2 solution 10-fold.
3. Prepare H_2O_2 Standards according to Table 2. Mix well.

Table 2.

Preparation of H_2O_2 Standards for Fluorometric assay

Well	0.1 mM H_2O_2 Standard	Catalase Assay Buffer	Stop Solution	H2O2 (nmol/well)
1	0 µL	90 µL	10 µL	0
2	2 µL	88 µL	10 µL	0.2
3	4 µL	86 µL	10 µL	0.4
4	6 µL	84 µL	10 µL	0.6
5	8 µL	82 µL	10 µL	0.8
6	10 µL	80 µL	10 µL	1

Assay Procedure

1. Add 12 µL of freshly prepared 1 mM H_2O_2 (see Steps 1 and 2 of the Colorimetric Standard Curve Preparation) into each well (samples, positive control, and sample HC) to start the reaction.
2. Incubate at 25 °C for 30 minutes. Samples with low concentrations of catalase may require longer than 30 minutes. Continue incubation until sufficient color develops, noting incubation time (required for the results calculation).
3. Add 10 µL of Stop Solution into each Sample and Positive Control well to stop the reaction. **Do not add** Stop Solution to the High Control (HC) or standard curve wells as they already contain Stop Solution.
4. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Developer Reaction Mix according to Table 3 (Colorimetric) or Table 4 (Fluorometric).

Table 3.

Preparation of Colorimetric Developer Reaction Mix

Reagent	Volume
Catalase Assay Buffer	46 µL
Probe	2 µL
HRP Solution	2 µL

Table 4.

Preparation of Fluorometric Developer Reaction Mix

Reagent	Volume
Catalase Assay Buffer	48.2 µL
Probe	0.3 µL
HRP Solution	1.5 µL

Add 50 µL of the appropriate Developer Mix to each test sample, controls, and standard wells. Mix well and incubate at 25 °C for 10 minutes.

Measurement

Measure at 570 nm (A_{570}) for colorimetric detection or λ_{Ex} = 535 nm/λ_{Em} = 587 nm for fluorometric detection in a plate reader.

Results

1. Signal change by catalase in the sample is $\Delta A = A_{HC} - A_{sample}$, where A_{HC} is the absorbance reading of the sample High Control and A_{sample} is the absorbance reading of the sample at 30 minutes.
2. Plot the H_2O_2 Standard Curve.
3. Apply the ΔA to the H_2O_2 standard curve to get B nmol of H_2O_2 decomposed by catalase in the 30 minute reaction.
4. Calculate catalase activity:

Catalase activity (nmol/min/mL = mU/mL) =

$$\frac{B}{T \times V} \times \text{Sample Dilution Factor}$$

Where:

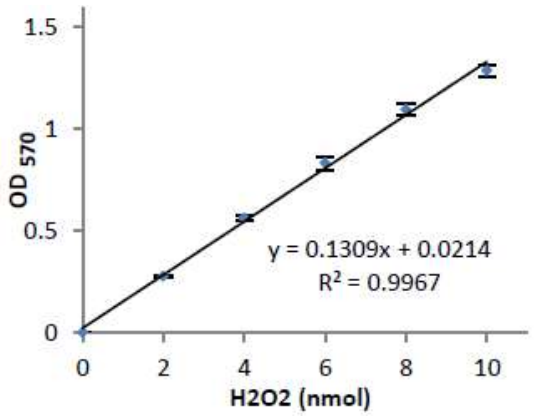
B = the decomposed H_2O_2 amount from H_2O_2 Standard Curve (in nmol).

V = the pretreated sample volume added into the reaction well (in mL).

T = Reaction time. 30 minutes is the standard time per the procedure unless the time was increased for low catalase concentrations. See Assay Procedure Step 2.

Unit definition: One unit of catalase is the amount of catalase that decomposes 1.0 µmol of H_2O_2 per minute at pH 4.5 at 25 °C.

Figure 1.



Typical H_2O_2 standard curve (colorimetric)

Frequently Asked Questions

What is the sensitivity of this assay?

The lower end of sensitivity is high pico-units to 1 microunit (μU) of Catalase activity.

Is it essential to make a standard curve for every assay run, or is one curve per kit enough?

It is strongly recommended to prepare a standard curve every time an assay is run due to varying conditions from run to run.

Can plasma/whole blood samples be processed for this assay?

The technical bulletin contains instructions for Erythrocytes. Whole blood can be processed similarly. Plasma can be diluted over a range and then the dilution that gives readings within the linear range of the standard curve can be used for the assay.

What is the activity level of the positive control? How can we increase its value to be comparable with our samples?

The positive control is only a benchmark sample. As long as the values are within the range of the standard curve, this is fine. The positive control is not be used to compare values with the samples. The positive control is provided to validate that the assay components are all working. The user can add more volume to get higher values, but this is not necessary as long as the values are within the standard curve range. Catalase is a very vulnerable enzyme to freeze-thaw and can lose activity with storage over time.

Is sonication enough to lyse cells?

It is recommended to homogenize cells using a Dounce tissue grinder set. This method is gentle yet effective. If needed, sonication can be used in addition to homogenization. Please note that generation of heat during sonication affects the activity of catalase enzyme.

Do bacterial samples work with this kit?

The kit was developed with mammalian samples, but it does work with a variety of samples from different species including bacteria. For gram positive bacteria, lysis reagents (Lysozyme treatment) might be required to rupture the cell wall. Gram negative bacterial cells can simply be homogenized as described in the protocol. It is recommended to test different volumes/dilutions of the samples to ensure the final readings are within the linear range of the standard curve.

Can food samples be used?

Food samples can be homogenized with the assay buffer and then centrifuged to collect the supernatant which will then be the sample for the assay. Liquid food samples can be tested without any preparation. All samples must be centrifuged to ensure there is no floating debris or particulate material. It is recommended to test several volumes of the samples to optimize the amount needed to ensure the final readings are within the linear range of the standard curve.

The RFU values are the same for increasing volumes of our sample. Why?

The classic cue to saturation is that when you add more sample the values decrease, meaning the maximum has already been attained and there is limitation of either reagents or V_{max} has been reached already. When there is a very high amount of catalase in the sample, all the substrate is quickly converted into product and then substrate is no longer available limiting the color development. When the sample is diluted, there is less catalase and hence the substrate is gradually converted to product showing a gradual increase over time. Sample volume needs to be optimized to ensure that values in the linear range of the standard curve are obtained.

Can alternate buffers be used for sample preparation (cell lysis, sample dilutions, etc.)?

The Catalase Assay Kit assay buffer is optimized for the reaction. The assay buffer not only contains some detergents for efficient lysis of cells/tissue, but also contains proprietary components required for the further reactions. Therefore, it is highly recommended to only use the buffer provided in the kit for the best results.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.