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Product Information

Catalase Assay Kit

Catalog Number **CAT100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Catalase is an antioxidant enzyme ubiquitously present in mammalian and non-mammalian aerobic cells containing a cytochrome system. It was initially isolated from ox liver and later from blood, bacterial, and plant sources.[1] The enzyme contains 4 ferrihemoprotein groups per molecule. The enzyme has a molecular mass of 240 kDa. Catalase activity varies greatly between tissues. The activity is highest in the liver and kidney, and lowest in connective tissues. In eukaryotic cells the enzyme is concentrated in the subcellular organelles called peroxisomes (microbodies).[2]

Catalase catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in the eukaryotic cell as a by-product of various oxidase and superoxide dismutase reactions. Hydrogen peroxide is highly deleterious to the cell and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death.[5-8] Removal of the H_2O_2 from the cell by catalase provides protection against oxidative damage to the cell. Its role in oxidative stress related diseases has been widely studied.[5,9]

This kit provides a simple and easy colorimetric assay for the study of catalase activity in various tissues and subcellular organelles.[3,4] It also provides a direct UV assay for catalase samples that do not contain UV interfering substances.

Reagents

This kit provides reagents sufficient for 100 tests.

- Assay Buffer 10× 100 ml
  (Catalog Number A9725)
  500 mM potassium phosphate buffer, pH 7.0

- Chromogen Reagent 1 vial
  (Catalog Number C5237)

- Stop Solution 100 ml
  (Catalog Number S5691)
  15 mM sodium azide in water

- Catalase Positive Control 0.25 ml
  (Catalog Number C8362)
  from bovine liver (EC 1.11.1.6)
  Crystalline suspension in water containing 0.1% thymol
  30–50 mg protein per ml
  3–10 × 10^6 units per ml (as measured using the colorimetric assay in this kit)

  Unit definition: One unit of catalase will decompose 1.0 micromole of hydrogen peroxide to oxygen and water per minute at pH 7.0 at 25 °C at a substrate concentration of 50 mM hydrogen peroxide.

- 3% (w/w) Hydrogen Peroxide Solution 10 ml
  (Catalog Number 323381)

- Peroxidase 5 mg
  (Catalog Number P6782)
  from horseradish (EC 1.11.1.7)
  Essentially salt free.
  800–1,200 units per mg solid as measured with ABTS as the substrate at 25 °C at pH 5.0

- Enzyme Dilution Buffer 100 ml
  (Catalog Number E5779)
  50 mM potassium phosphate buffer, pH 7.0, containing 0.1% Triton™ X-100

Reagents and Equipment Required but not Provided

- Spectrophotometer
- 1 ml cuvette (quartz is required for UV Assay method)
- Analytical balance
- Ultrapure water
- Beaker (250 ml)
- Bovine serum albumin, Catalog Number A8022

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.

Preparation Instructions
Use ultrapure water in all cases. The preparation instructions will provide reagents sufficient for 25 colorimetric assays plus a calibration curve or 20 UV assays.
Note: The substrate and buffer solutions should be kept at room temperature and the enzyme solutions at 4 °C.

1× Assay Buffer: Dilute 2 ml of the Assay Buffer 10× 10-fold to 20 ml with water. The 1× Assay Buffer is 50 mM potassium phosphate buffer, pH 7.0. Store at room temperature.

Peroxidase Solution: Weigh 1 mg of solid Peroxidase and dissolve in 1.45 ml of 1× Assay Buffer. The peroxidase solution can be stored at 4 °C for up to 2 weeks.

Chromogen Solution: Mix 60 ml of Assay Buffer 10× with 140 ml of water in a 250 ml beaker. Add 10 ml of diluted buffer to the Chromogen Reagent vial and mix until completely dissolved. Transfer the dissolved Chromogen Reagent from the vial into the buffer-containing beaker and mix well. Divide into suitable aliquots and store at –20 °C. The prepared Chromogen Solution is stable for 12 months. Avoid multiple freeze-thaw cycles.

Color Reagent (150 mM potassium phosphate buffer, pH 7.0, containing 0.25 mM 4-aminoantipyrine and 2 mM 3,5-dichloro-2-hydroxybenzenesulfonic acid): Before use prepare the Color Reagent by adding 30 µl of the Peroxidase Solution to 30 ml of Chromogen Solution. The Color Reagent may be kept at 4 °C for three days if necessary.

Catalase Control: Catalase (Catalog Number C8362) is a crystalline suspension in water and the crystals precipitate to the bottom of the tube. Vortex the tube of catalase vigorously to obtain a homogenous suspension and immediately remove 20 µl of the suspension. It is recommended to pipette up and down several times before removing the suspension. Serially dilute the 20 µl of the enzyme suspension 10,000-fold [An example of this dilution is to dilute the 20 µl of the enzyme suspension with Enzyme Dilution Buffer to 400 µl (1:20), then dilute 20 µl of the first diluted solution to 400 µl (1:400), and finally, dilute 20 µl of the second solution to 500 µl (1:10,000)]. Use between 2–5 µl of the final dilution per reaction. Vortex well before adding to the reaction mixture. Prepare the Catalase Control fresh each day. The assay is linear in the range of 0.25–3 units per reaction mixture depending on the length of the reaction time.

Colorimetric Assay Substrate Solution (200 mM H_2O_2): The concentration of 3% H_2O_2 is in the range of 3–4%. Thus, it is vital to determine the exact concentration spectrophotometrically and correct it to 200 mM before using the Colorimetric Assay Substrate Solution in the assay. Dilute 200 µl of the 3% H_2O_2 to 1 ml with 1× Assay Buffer. In order to determine the exact concentration of the Substrate Solution, dilute 50 µl of the above solution to 1 ml (20-fold) with 1× Assay Buffer. The expected concentration is in the range of 10–15 mM. Determine the actual concentration by UV absorbance by measuring the absorbance at 240 nm (1× Assay Buffer is used as the blank). Calculate the actual H_2O_2 concentration using Beer's Law ($\varepsilon^{mM} = 0.0436$):

$$[H_2O_2]\ (mM) = \frac{A_{240}}{0.0436}$$

Adjust the final concentration of the Colorimetric Assay Substrate Solution to exactly 200 mM with 1× Assay Buffer. The standardized Colorimetric Assay Substrate Solution may be stored for 6 days at 4 °C. The final concentration of H_2O_2 in the assay mixture is 50 mM.

10 mM H_2O_2 Solution: This solution is for obtaining a standard curve of the absorbance of the red quinoneimine dye versus H_2O_2 concentration. Dilute 200 µl of the standardized Colorimetric Assay Substrate Solution (200 mM H_2O_2) to 4 ml with 1× Assay Buffer. This solution may be stored for 6 days at 4 °C.

UV Assay Substrate Solution (20 mM H_2O_2): Preparation of volume sufficient for 20 Direct UV assays. Dilute 200 µl of the 3% H_2O_2 (Catalog Number 323381) to 10 ml with 1× Assay Buffer. Determine the actual concentration spectrophotometrically (use Beer's Law). Adjust the final concentration of the UV Assay Substrate Solution to exactly 20 mM with 1× Assay Buffer. The standardized UV Assay Substrate Solution may be stored for 6 days at 4 °C. The final concentration of H_2O_2 in the assay mixture is 10 mM.

Sample preparation: When samples are prepared by lysis with detergent or hypotonic buffer, 1× Assay Buffer can be used to dilute the samples. If the sample is prepared in an isotonic buffer that keeps the peroxisome intact, dilute the sample with the Enzyme Dilution Buffer that contains 0.1% Triton X-100. If the protein concentration of the sample is very low (<0.025 mg/ml), bovine serum albumin (Catalog Number A8022) may be added to the buffer at a concentration of 0.5 mg/ml to stabilize the enzyme.

For biological samples the amount of enzyme (dilution and volume) per reaction has to be determined. Table 1 gives examples of dilutions and volumes for various samples, which may be used as guidelines. Although most reactions can be performed in 1–5 minutes, the reaction time may be extended greatly to assay for low catalase activities.

When assaying tissue extracts, the amount of catalase will depend on the organ source of the tissue. The activity is highest in liver and kidney, and lowest in connective tissues. Cell extracts from tissue culture are often assayed without dilution. Blood lysates show a relatively high amount of activity and therefore should be diluted accordingly. See Table 1 for recommended concentrations and reaction times for various sample types.

Table 1.
Dilutions of catalase samples from various sources

Tissue	Dilute Sample to following protein conc.	Volume added to Reaction Mixture	Reaction Time (minutes)	Range of ΔA_{520} (blank – sample) per Reaction
Blank	–	–	–	1.20–1.32 (initial A_{520})
Human red blood cell lysate	0.2 mg/ml	2–6 µl	2 min	0.21–0.71
Jurkat lysate	2.0 mg/ml	2–4 µl	3 min	0.28–0.64
HepG2 lysate	2.0 mg/ml	2–4 µl	3 min	0.21–0.43
Rat liver lysate	0.3 mg/ml	5–10 µl	1 min	0.36–0.78
Rat brain lysate	0.7 mg/ml	10–20 µl	3 min	0.014–0.028
Rat spleen lysate	0.3 mg/ml	5–10 µl	3 min	0.11–0.21
Rat kidney lysate	0.3 mg/ml	5–10 µl	3 min	0.28–0.57
Catalase standard	10,000-fold	2–4 µl	1 min	0.36–0.71

If there is no information available for a source, it is recommended to prepare several dilutions (1, 10, 20, and 50-fold dilutions) and run a 1 minute reaction with 10 µl of each dilution. The dilution recommended should decrease the concentration of H_2O_2 in the reaction by 30–50% in 1–5 minutes.

Storage/Stability
This kit ships on wet ice and it is recommended to store the kit at 2–8 °C. When stored unopened, the components in this kit remain active for 24 months.

Procedures for Colorimetric and UV Assays
Principle of assays
Catalase is able to decompose hydrogen peroxide by two different reaction pathways. In the first, known as the "catalatic" pathway, 2 molecules of hydrogen peroxide are converted to water and oxygen (catalatic activity):[1]

$$\text{Protein-Fe}^{3+} + H_2O_2 \rightarrow \underset{\text{(Primary Complex)}}{\text{Protein-Fe}^{3+}\text{-OOH}} + H_2O$$

$$\text{Protein-Fe}^{3+}\text{-OOH} + H_2O_2 \rightarrow \text{Protein-Fe}^{3+}\text{-OH} + H_2O + O_2$$

The overall reaction gives: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

The primary complex can also decompose by another pathway (peroxidatic decomposition):[2]

$$\text{Protein-Fe}^{3+}\text{-OOH} + AH_2 \rightarrow \text{Protein-Fe}^{3+}\text{-OH} + H_2O + A$$

where AH_2 is an internal or external donor of hydrogen. Low molecular weight alcohols can serve as electron donors. The catalatic pathway is predominant when the hydrogen peroxide concentration is >0.1 mM and the peroxidatic pathway is dominant when the hydrogen peroxide concentration is <0.1 mM or the substrate is an alkyl peroxide.

Colorimetric Assay Procedure
This assay method is based on the measurement of the hydrogen peroxide substrate remaining after the action of catalase. First, the catalase converts hydrogen peroxide to water and oxygen (catalatic pathway) and then this enzymatic reaction is stopped with sodium azide. An aliquot of the reaction mix is then assayed for the amount of hydrogen peroxide remaining by a colorimetric method.[10] The colorimetric method uses a substituted phenol (3,5-dichloro-2-hydroxybenzene-sulfonic acid), which couples oxidatively to 4-aminoantipyrine in the presence of hydrogen peroxide and horseradish peroxidase (HRP) to give a red quinoneimine dye (N-[4-antipyryl]-3-chloro-5-sulfonate-*p*-benzoquinone-monoimine) that absorbs at 520 nm.

Catalase activity is measured at a non-saturating substrate (H_2O_2) concentration, since it is not feasible to saturate the enzyme with the substrate at greater than 1 M.[11] Also, above 100 mM H_2O_2 there is a rapid inactivation of the catalase by the substrate. The concentration of hydrogen peroxide used in this assay (50 mM) provides a measurable signal but does not cause inactivation of the enzyme.

Preparation of Standard Curve - Absorbance of the Red Quinoneimine Dye versus amount of H_2O_2 (0.0125–0.075 µmole)

1. Prepare a series of standard solutions of H_2O_2 by placing 0, 125, 250, 500, and 750 µl of 10 mM H_2O_2 solution in microcentrfuge tubes and adding 1× Assay Buffer to a final volume of 1.0 ml (see Table 2). Mix by inversion.

Table 2.
Dilutions for Preparation of the Hydrogen Peroxide Standard Curve

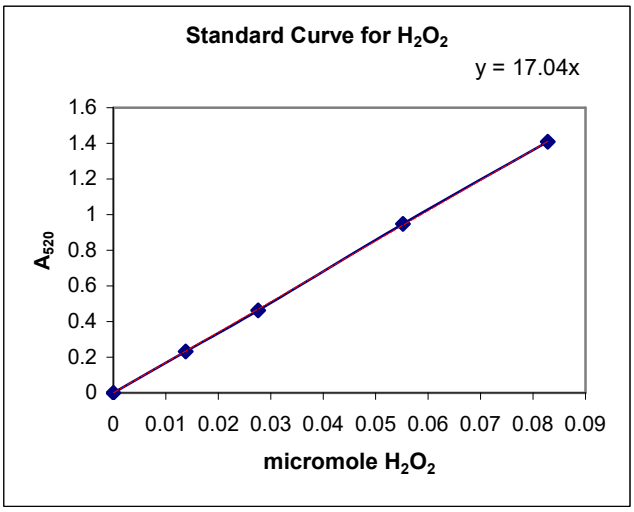
Volume of 10 mM H_2O_2	1× Assay Buffer	H_2O_2 in standard solution (mM)	H_2O_2 in Reaction Mixture* (mM)
0 µl	1,000 µl	0	0
125 µl	875 µl	1.25	0.0125
250 µl	750 µl	2.5	0.0250
500 µl	500 µl	5.0	0.0500
750 µl	250 µl	7.5	0.0750

*Note: The H_2O_2 concentrations shown in Table 2 are based on an exact 10 mM concentration of the starting solution. The values should be corrected for the actual concentration of H_2O_2 found spectrophotometrically (see Preparation Instructions, 10 mM H_2O_2 Solution).

2. Transfer a 10 µl aliquot of each standard solution to a second tube and add 1 ml of the Color Reagent. Wait 15 minutes and then read the absorbance at 520 nm.
   Note: The series of standard solutions of H_2O_2 should be prepared fresh each day.

3. Plot a standard curve of the absorbance at 520 nm versus the final amount of H_2O_2 in the reaction mixture (see Figure 1).

Figure 1.
Example of a Standard Curve of the Absorbance of the Red Quinoneimine Dye versus amount of H_2O_2



Colorimetric Assay Reaction
The assay reaction is performed at room temperature (~25 °C). Allow the 1× Assay Buffer, Colorimetric Assay Substrate Solution (200 mM H_2O_2), and Color Reagent to equilibrate to room temperature.

Catalase Enzymatic Reaction (see Table 3):
1. Prepare the sample as suggested under Preparation Instructions, Sample Preparation, and add the appropriate volume (x µl) to a microcentrifuge tube.
2. Add (75–x) µl of 1× Assay Buffer to the microcentrifuge tube.
3. Start the reaction by addition of 25 µl of the Colorimetric Assay Substrate Solution.
4. Mix by inversion and incubate 1–5 minutes.
5. Add 900 µl of the Stop Solution and invert the tube.

Table 3.
Catalase Colorimetric Enzymatic Reaction Scheme

	Sample Volume	1× Assay Buffer	200 mM H_2O_2 Solution
Blank	0	75 µl	25 µl
Sample	x µl	75–x µl	25 µl

Colorimetric Reaction:
6. Remove a 10 µl aliquot of the Catalase Enzymatic Reaction mixture from Step 5 and add to another microcentrifuge tube. Add 1 ml of the Color Reagent. Mix by inversion.
   Note: Perform this step within 15 minutes of stopping the enzymatic reaction.

7. Wait at least 15 minutes at room temperature for color development and measure the absorbance at 520 nm.

Calculations

1. Determine the amount of H_2O_2 (μmoles) remaining in the Colorimetric Reaction mixture using the H_2O_2 standard curve (see Figure 1). For example, an OD_{520} of 1.4 is equivalent to 0.082 μmole of H_2O_2.

   A_{520}(Blank) = μmoles of H_2O_2 in Blank
   A_{520}(Sample) = μmoles of H_2O_2 in Sample

   Δμmoles (H_2O_2) = μmoles of H_2O_2 (Blank) – μmoles of H_2O_2 (Sample)

   Δμmoles (H_2O_2) is the difference in amount of H_2O_2 added to the Colorimetric Reaction between the Blank and a given Sample.

2. The value from calculation 1 can be used to determine the catalase activity:

   $$\text{Activity (μmoles/min/ml)} = \frac{\Delta\text{μmoles } (H_2O_2) \times d \times 100}{V \times t}$$

   Δμmoles (H_2O_2) = difference in amount of H_2O_2 added to the Colorimetric Reaction between the Blank and a given Sample
   d = dilution of original sample for Catalase Enzymatic Reaction
   t = Catalase Enzymatic Reaction duration (minutes)
   V = sample volume in Catalase Reaction (x μl = 0.00x ml)
   100 = dilution of aliquot from Catalase Enzymatic Reaction in Colorimetric Reaction (10 μl from 1 ml)

Unit definition: One unit of catalase will decompose 1.0 micromole of hydrogen peroxide to oxygen and water per minute at pH 7.0 at 25 °C at a substrate concentration of 50 mM hydrogen peroxide.

UV Assay Procedure

This kit may also be used to perform a quick and direct UV assay. The UV assay is suitable for catalase samples free of substances that may interfere with the UV measurement at 240 nm (see Table 5). Interfering substances such as Triton X-100 or proteins, which absorb in the UV region, must be present at concentrations low enough to allow monitoring the absorbance at 240 nm.

The assay involves spectrophotometrically following the decrease in absorbance of hydrogen peroxide at 240 nm with a kinetic program.[12] The assay is performed with a short time period (30 seconds), due to the fact that the H_2O_2 concentration (10 mM) is much lower than the K_M (1.2 M) of the system.[11]

The assay reaction is performed at room temperature (~25 °C). The kinetic program has the following parameters:

Initial delay = 3 seconds
interval = 5 seconds
readings = 7

Catalase Enzymatic Reaction (see Table 4):

1. Prepare the sample as suggested under Preparation Instructions, Sample Preparation and add the appropriate volume (x μl) to a quartz cuvette.
2. Add (500–x) μl of 1× Assay Buffer to the quartz cuvette and mix by inversion.
3. Start the reaction by adding of 0.5 ml of UV Assay Substrate Solution (20 mM H_2O_2) and mix by inversion
4. Follow the decrease of A_{240} for 30 seconds with the kinetic program.
   Notes: The initial A_{240} should be ~0.500. Use the buffer in which the sample was diluted as the blank. The concentration of Triton X-100 in the assay should not exceed 0.02%. The reliable detection limit is 0.025 ΔA_{240}/minute, which is equal to 0.575 μmole/minute.

Table 4.
Catalase UV Enzymatic Reaction Scheme

	Sample Volume	**1x Assay Buffer**	**20 mM H_2O_2 Solution**
Blank	0	500 μl	500 μl
Sample	x μl	500–x μl	500 μl

Calculation

$$\text{Units/ml} = \frac{[\Delta A/\text{min(Blank)} - \Delta A/\text{min(Sample)}] \times d \times 1}{V \times 0.0436}$$

d = dilution of original sample for Catalase Enzymatic Reaction
V = sample volume in Catalase Enzymatic Reaction, (x µl = 0.00x ml)
0.0436 = ε^{mM} for hydrogen peroxide
1 = reaction volume in ml

Unit definition: One unit of catalase will decompose 1.0 micromole of hydrogen peroxide to oxygen and water per minute at pH 7.0 at 25 °C at a substrate concentration of 10 mM hydrogen peroxide.

Note: The activity measured by the UV Assay Procedure is approximately one third that measured with the Colorimetric Assay Procedure. This is due to differences in the substrate concentrations (20 mM in the UV assay versus 50 mM in the colorimetric assay)

Compatibility Chart

Various compounds may interfere with the assays (see Table 5). Since blood is one of the sources for catalase activity, anticoagulants such as sodium citrate, potassium EDTA, or heparin have been investigated for their effect on the assay. Also, endogenous compounds such as hemoglobin or albumin will have an effect on the assay. Normally the sample is diluted considerably before assaying; however, in tissues with low catalase content these compounds may interfere due to a small dilution factor. In addition, there may be an advantage to use the UV assay procedure if the interfering substance severely affects the colorimetric assay procedure.

Table 5.
Effect of Substances on Colorimetric and UV assays

Substance	Colorimetric Assay	UV Assay	Expected Concentration in Undiluted Sample
Ascorbic acid	20% inhibition at 20 µM	Compatible up to 100 µM	29 µM in RBC; 77 µM in plasma
Albumin, bovine	Compatible at 50 mg/ml	Compatible at 1.5 mg/ml	35–50 mg/ml in blood
Sodium citrate	Compatible at 20 mM	Compatible at 5 mM	10–14 mM in blood
Tripotassium EDTA	Compatible at 4 mM	Compatible at 1 mM	3.4 mM in blood
Hemoglobin	Compatible at 0.8 mg/ml	Compatible at 1 mg/ml	120–180 mg/ml in blood
Heparin	Compatible at 14 units/ml	Compatible at 20 units/ml	1 unit/ml in blood
Glucose	Compatible to 5 mM	Compatible to 50 mM	17–44 mM in blood
Triton X-100	Compatible to 0.5%	Compatible to 0.02%	Not normally present

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