Catalase Colorimetric Activity Kit

Catalog Number EIACATC (192 tests)

Rev 2.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Catalase Colorimetric Activity Kit is an activity assay designed to measure catalase activity in serum, plasma, tissue lysates, cell lysates, or erythrocyte lysates. The assay was characterized with human catalase, but is expected to measure catalase activity in samples from other species.

Catalaseis a highly conserved enzyme expressed in all mammalian tissues, with high levels of catalase found in the liver, kidneys and erythrocytes. The enzyme catalyzes the hydrolysis of hydrogen peroxide into water and oxygen, to prevent the potential harmful effects of excessive levels of hydrogen peroxide.

Contents and storage

Kit and components are shipped at 4°C. Upon receipt, store the kit at 4°C until the expiration date on the kit box. DO NOT FREEZE.

Components	Quantity
CatalaseStandard; 100 U/mL bovine catalase in a special solution	90 µL
Assay Buffer Concentrate (5X)	25 mL
Clear 96-well Half Area Plate	2 plates
Hydrogen Peroxide Reagent; contains very dilute H_2O_2	5 mL
Substrate	5 mL
Horseradish Peroxidase Concentrate (50X)	120 µL

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 560 nm, with excitation at 390 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare samples

Sample	Procedure
Cell lysate	1. Prepare cell pellet • Centrifuge > 1 × 10^6 **cells in suspension** at 250 × *g* for 10 minutes at 4°C. Discard the supernatant. • Remove media from plate with **adherent cells** and gently dislodge the cells with a rubber policeman. Do not add proteolytic enzymes. 2. Homogenize or sonicate the pellet in 1–2 mL of cold 1X Assay Buffer per 100 mg of cells. 3. Centrifuge at 10,000 × *g* for 15 minutes at 4°C. Collect the supernatant and assay immediately, or store at ≤-70°C.
Tissue lysate	1. Wash tissue thoroughly with ice cold PBS prior to processing to remove any red blood cells or clots. 2. Homogenize or sonicate the tissue in 0.5–1 mL of cold 1X Assay Buffer per 100 mg of tissue. 3. Centrifuge at 10,000 × *g* for 15 minutes at 4°C. Collect the supernatant and assay immediately, or store at ≤-70°C.
Serum	1. Collect serum in tubes without anticoagulant. Allow to clot for 30 minutes at room temperature. 2. Centrifuge the sample at 2,000 × *g* for 15 minutes at 4°C. 3. Collect the pale yellow serum without disturbing the white buffy layer and transfer to a clean tube. Assay immediately, or store at ≤-70°C.
Plasma and erythrocytes	1. Collect plasma in tubes with EDTA or heparin anticoagulant. 2. Centrifuge the sample at 700–1,000 × *g* for 10 minutes at 4°C. 3. Collect the pale yellow plasma without disturbing the white buffy layer and transfer to a clean tube. 4. Remove and discard the white buffy layer. 5. Add 4 volumes of ice cold deionized water to lyse erythrocytes. 6. Centrifuge at 10,000 × *g* for 15 minutes at 4°C. Collect the supernatant and assay immediately, or store at ≤-70°C.

Dilute samples

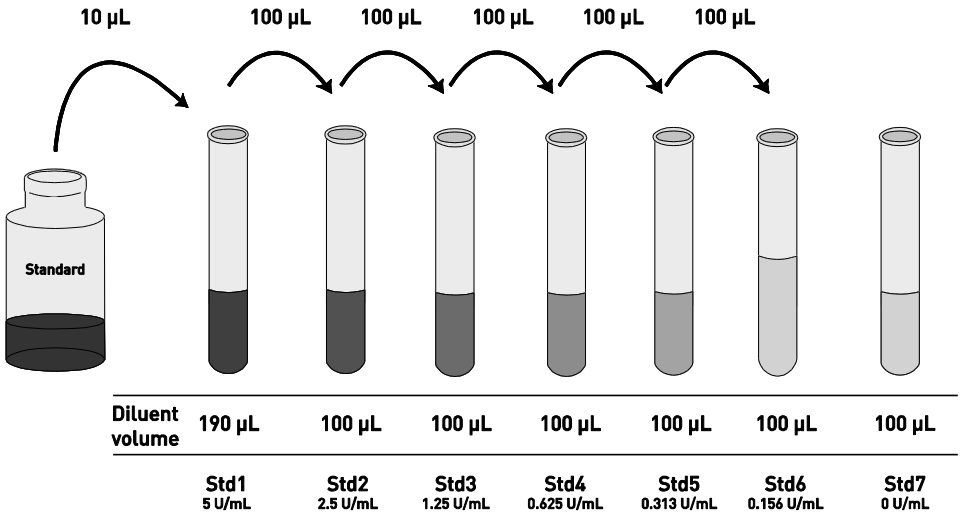
Sample activity should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions in 1X Assay Buffer.
- Dilute **serum** samples ≥1:5 in 1X Assay Buffer.
- Dilute **plasma and erythrocyte** samples ≥1:10 in 1X Assay Buffer.
- Use all samples within **2 hours** of dilution.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.
Note: One unit of catalase decomposes 1.0 µmol of H_2O_2 per minute at pH 7.0 and 25°C.

1. Add 10 µL Catalase Standard to one tube containing 190 µL 1X Assay Buffer and label as 5 U/mL catalase.
2. Add 100 µL 1X Assay Buffer to each of 6 tubes labeled as follows: 2.5, 1.25, 0.625, 0.313, 0.156, and 0 U/mL catalase.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Prepare 1X HRP solution

Prepare 1X HRP solution according to the following table. The 1X HRP solution is stable for one day.

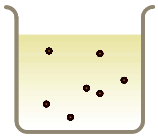
Note: Vortex the Horserasdish Peroxidase Concentrate (50X) prior to pipetting. Pipet from the bottom of the tube.

Reagent	½ plate	1 plate	1½ plates	2 plates
Horserasdish Peroxidase Concentrate (50X)	27 µL	50 µL	76 µL	100 µL
1X Assay Buffer	1.323 mL	2.45 mL	3.724 mL	4.9 mL
Total volume	1.35 mL	2.5 mL	3.8 mL	5 mL

Assay procedure

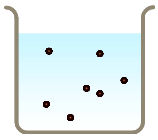
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 45 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample and peroxide

a. Add 25 µL of standards or diluted samples (see page 2) to the appropriate wells.
b. Add 25 µL of Hydrogen Peroxide Reagent into each well.
c. Incubate for 30 minutes at room temperature.



Add substrate and 1X HRP

a. Add 25 µL of Substrate into each well.
b. Add 25 µL of 1X HRP solution into each well
c. Incubate for 15 minutes at room temperature.

Read the plate and generate the standard curve

1. Read the absorbance at 560 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the activity of unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals lower than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the activity by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–5.0 U/mL catalase.

Standard catalase (U/mL)	Optical Density (500 nm)
5.0	0.092
2.5	0.388
1.25	0.858
0.625	1.283
0.313	1.525
0.156	1.676
0	1.811

Intra-assay precision

Three human samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	1.71	0.84	0.48
%CV	3.5	4.0	4.8

CV = Coefficient of Variation

Inter-assay precision

Three human samples were assayed 21 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	1.79	0.94	0.53
%CV	11.9	9.8	12.3

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Five random adult human serum and plasma samples were diluted in Assay Buffer between 1:10 and 1:80 and run in the assay.

Sample	Range (U/mL)	Average (U/mL)
Serum	6.04–128.3	36.9
Plasma	11.4– 157.1	70.2

Linearity of dilution

Linearity was determined by assaying serum samples with high and low concentrations of catalase (high sample 1.163 U/mL; low sample 0.485 U/mL) mixed in the ratios shown in the following table.

High Sample %	Low Sample %	Expected Conc. (mU/mL)	Observed Conc. (mU/mL)	% Recovery
80	20	1.027	1.058	103.0
60	40	0.892	0.952	106.8
40	60	0.756	0.665	87.9
20	80	0.621	0.593	95.6
			Mean Recovery	**98.3%**

Sensitivity

The analytical sensitivity of the assay is 0.052 U/mL catalase. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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Catalog Number

Batch code


Temperature limitation


Use by


Manufacturer


Consult instructions for use


Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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