

Catalase Assay Kit

Cat. No. Kit-2435 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	CATALASE (EC 1.11.1.6), is an ubiquitous antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H ₂ O ₂) to water and oxygen. By preventing excessive H ₂ O ₂ build up, catalase allows important cellular processes which produce H ₂ O ₂ as a byproduct to occur safely. Deficiency in catalase activity has been associated with grey hair and peroxisomal disorder acatalasia. Simple, direct and high-throughput assays for catalase activity find wide applications. improved assay directly measures catalase degradation of H ₂ O ₂ using a redox dye. The change in color intensity at 570nm or fluorescence intensity ($\lambda_{ex/em}$ = 530/585nm) is directly proportional to the catalase activity in the sample.
Applications	Direct Assays: catalase activity in biological samples e.g. serum, plasma, urine, saliva, cell culture etc. Drug Discovery/Pharmacology: effects of drugs on catalase activity.
Stability	Shelf life of 6 months after receipt.
Storage	-20°C
Shipping	On Ice
Size	100 tests
Kit Components	Assay Buffer: 25 mL HRP Enzyme: 120 μ Dye Reagent: 120 μ

 Tel: 1-631-559-9269 1-516-512-3133

 Email: info@creative-biomart.com  Fax: 1-631-938-8127

 45-1 Ramsey Road, Shirley, NY 11967, USA

	<p>H2O2 Solution: 100 μ 3% H2O2</p> <p>Positive Control: 8 μ Catalase</p>
Detection method	OD570nm, or FL530/585nm
Compatible Sample Types	Serum, plasma, urine, saliva, cell culture etc
Features & Benefits	<p>Sensitive and accurate. Use 10 μL sample. Linear detection range 0.2 to 5 U/L catalase activity.</p> <p>Simple and Convenient. The procedure involves adding a Substrate to sample, incubation for 30 min, followed by a Detection Reagent and reading the optical density or fluorescence intensity.</p>
Preparation	<p>SAMPLE PREPARATION</p> <p>Tissue (10 mg) and cells (10^6) are homogenized in 200 μ cold PBS. Centrifuge 10 min at 14,000 rpm to pellet any debris. Use clear supernatant for assay.</p> <p>Note: SH-containing reagents (e.g. β-mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be kept below 10 μM in the sample.</p>
Assay Protocol	<p>1. Reagent Preparation. Equilibrate all components to room temperature. Briefly centrifuge all tubes before opening. Keep thawed HRP Enzyme on ice.</p> <p>For colorimetric assays, use a clear flat-bottom 96-well plate. For fluorimetric assays, use a solid black flat-bottom 96-well plate.</p> <p>Samples and Controls: transfer 10 μ sample into wells of the 96-well plate. In addition, for each assay run, prepare one sample blank well that contains only 10 μ Assay Buffer.</p> <p>Add 400 μ Assay Buffer to Positive Control tube and mix well. Transfer 10 μ of the reconstituted Positive Control into separate wells.</p> <p>Note: (1). For unknown samples, perform several dilutions to ensure that catalase</p>

activity is within the linear range 0.2 to 5 U/L. (2) The provided catalase serves as a positive control to ensure assay is working and should not be used to calculate the Sample catalase activity.

2. Enzyme Reaction. Mix 5 μ 3% H₂O₂ and 914 μ dH₂O (final 4.8 mM). Prepare enough 50 μ H₂O₂ Substrate for sample, positive control and sample blank by mixing, for each well, 1 μ of the 4.8 mM H₂O₂ with 95 μ Assay Buffer. Note: diluted H₂O₂ is not stable. Prepare fresh dilutions for each experiment.

Add 90 μ of the 50 μ Substrate to these wells to initiate the catalase reaction. Tap plate quick to mix. Incubate 30 min at room temperature. During the incubation time, proceed with Steps 3 and 4 below.

3. H₂O₂ Standard Curve. Mix 40 μ of the 4.8 mM H₂O₂ with 440 μ dH₂O to yield 400 μ H₂O₂. Prepare standards as shown in the Table below. Transfer 10 μ standards into separate wells of the 96-well plate. Add 90 μ Assay Buffer to the standards.

No 400 μ H₂O₂ + H₂O Vol (μ) H₂O₂ (μ)

1 100 μ + 0 μ 100 400

2 60 μ + 40 μ 100 240

3 30 μ + 70 μ 100 120

4 0 μ + 100 μ 100 0

4. Detection. Prepare enough Detection Reagent by mixing, for each reaction well (Sample, Control and Standard wells), 102 μ Assay Buffer, 1 μ Dye Reagent and 1 μ HRP Enzyme.

At the end of the 30 min incubation (Step 2), add 100 μ Detection Reagent per well. Tap plate to mix. Incubate for 10 min.

5. Read optical density at 570nm (550 to 585nm) or fluorescence intensity at λ em/ex = 585/530nm.

Assay time

40 min

Analysis

Subtract blank value (#4) from the standard values and plot the Δ OD or Δ F against

 Tel: 1-631-559-9269 1-516-512-3133

 Email: info@creative-biomart.com  Fax: 1-631-938-8127

 45-1 Ramsey Road, Shirley, NY 11967, USA

standard concentrations. Determine the slope and calculate the catalase activity of Sample,

$$\text{Catalase (U/L)} = (\text{R Sample Blank} - \text{R Sample}) / [\text{Slope (M-1)} \times 30 \text{ min}] \times n$$

R SAMPLE Blank and R SAMPLE are optical density or fluorescence intensity readings of the Sample Blank and Sample, respectively.

Slope is determined from the standard curve.

30 min is the catalase reaction time.

n is the sample dilution factor.

Unit definition: one unit is the amount of catalase that decomposes 1 μ mole of H₂O₂ per min at pH 7.0 and room temperature.

Sensitivity	0.2 U/L
--------------------	---------

 Tel: 1-631-559-9269 1-516-512-3133

 Email: info@creative-biomart.com  Fax: 1-631-938-8127

 45-1 Ramsey Road, Shirley, NY 11967, USA