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 (H2O2) to water and oxygen. By preventing excessive H2O2 build up, catalase allows important cellular processes which produce H2O2 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 H2O2 using a redox dye. The change in color intensity at 570nm or fluorescence intensity (λex/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	-20ºC
Shipping	On Ice
Size	100 tests
Kit Components	Assay Buffer: 25 mL HRP Enzyme: 120 µ Dye Reagent: 120 µ

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	H2O2 Solution: 100 µ 3% H2O2 Positive Control: 8 µ Catalase
Detection method	OD570nm, or FL530/585nm
Compatible Sample Types	Serum, plasma, urine, saliva, cell culture etc
Features & Benefits	Sensitive and accurate. Use 10 µL sample. Linear detection range 0.2 to 5 U/L catalase activity. 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.
Preparation	SAMPLE PREPARATION 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. 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.
Assay Protocol	1. Reagent Preparation. Equilibrate all components to room temperature. Briefly centrifuge all tubes before opening. Keep thawed HRP Enzyme on ice. For colorimetric assays, use a clear flat-bottom 96-well plate. For fluorimetric assays, use a solid black flat-bottom 96-well plate. 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. Add 400 µ Assay Buffer to Positive Control tube and mix well. Transfer 10 µ of the reconstituted Positive Control into separate wells. Note: (1). For unknown samples, perform several dilutions to ensure that catalase

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	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 µM 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 µM 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 40µ of the 4.8 mM H2O2 with 440 µ dH2O to yield 400 µM 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 µM H2O2 + H2O Vol (µ) H2O2 (µM) 1 100µ + 0µ 100 400 2 60µ + 40µ 100 240 3 30µ + 70µ 100 120 4 0µ + 100µ 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 λem/ex = 585/530nm.
Assay time	40 min
Analysis	Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against

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	standard concentrations. Determine the slope and calculate the catalase activity of Sample, Catalase (U/L) = (R Sample Blank – R Sample)/[Slope (μM-1) x 30 min] × 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 μmole of H_2O_2 per min at pH 7.0 and room temperature.
Sensitivity	0.2 U/L

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