BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Catalase Assay Kit (Cat #: E-100)

COMPONENTS: Catalase Substrate Solution- 25 ml, store at 4ºC (for 250 assays)
Catalase Assay Solution- 25 ml, store at room temperature
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: Catalase is present in nearly all aerobic cells and catalyzes the decomposition of H_2O_2 into oxygen and water. The enzyme is among the most efficient known, with rates approaching 200,000 catalytic events/second, which is near the diffusion-controlled limit. The assay kit is based on oxidation by H_2O_2 of an azo chromogen, causing appearance of a yellowish color with light absorption at 405 – 420 nm. Since the presence of catalase reduces the amount of H_2O_2 and inhibits the oxidation of the azo dye, the assay provides a semi-quantitative measurement of catalase activity in crude cell/tissue extracts. The kit is stable for at least one year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.1 – 1 mg/ml.

Enzyme assay:

1. Use a plain (uncoated) 96-well plate for the assay. Set up a "Control" well by adding 20 µl 1x Cell Lysis Solution to the well. Add 20 µl cell/tissue lysate to each "Sample" well.
2. Assay is initiated by adding 100 µl Catalase Substrate Solution to "Control" well and each "Sample" well. Gently agitate plate to mix contents. Cover and place plate in a 37ºC incubator for 5−20 min (to be determined by end users).
3. Stop reaction by adding 100 µl Catalase Assay Solution to each well. Agitate the plate briefly to mix contents. Cover and place plate in a humidified ~50ºC incubator for 30 min.

   Note: The assay can be performed in 0.5-ml microtubes for samples that exhibit precipitates after 50ºC incubation. Centrifuge tubes to remove precipitates and use supernatants for O.D. (absorbance) measurement.
4. Remove plate from incubator. Remove cover and allow plate to cool down for 10 min. Read O.D. at 405–420 nm using a plate reader. "Control" well should be the most yellowish well.

   Note: If sample well reading is similar to control well reading (low catalase activity), a more concentrated protein sample and/or longer reaction time at step 2 should be used for the assay. If sample well reading is lower than ~0.1 (high catalase activity), the sample should be diluted with 1x Cell Lysis Solution and re-assayed.

Calculation of catalase activity:

Sample catalase activity in optical unit = $\mathbf{O.D._{Control} - O.D._{Sample}}$

Enzyme activity may be presented as optical units/µg proteins. Alternatively, activity may be normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

- Avoid skin contact with Catalase Assay Solution, which contains 0.1N NaOH.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.
- Please visit the product webpage or contact us for SDS information.