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NWLSS™ Catalase Activity Assay

Product NWK-CAT01

For Research Use Only

Simple assay kit for quantitative measurement of catalase enzyme activity in biological samples . Includes stable standards eliminating the need for tedious H_2O_2 substrate calibrations.

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Introduction:
Hydrogen peroxide (H_2O_2) is formed in cells by controlled pathways and elicits a broad spectrum of cellular response ranging from mitogenic growth stimulation to apoptosis to necrosis at different concentration levels. Locally intense amounts of H_2O_2 can also be produced by inflammatory cells to kill pathogens. H_2O_2 at high concentration is deleterious to cells and its accumulation causes oxidation of cellular targets such as proteins, lipids and DNA leading to mutagenesis and/or cell death. Removal of H_2O_2 from cells is therefore necessary for protection against oxidative damage.

Catalase is an endogenous antioxidant enzyme present in all aerobic cells helping to facilitate the removal of hydrogen peroxide. The enzyme has four subunits of equal size, each containing a heme active site to promote the decomposition of H_2O_2 to water and oxygen.

Intended Use:
The NWLSS™ Catalase Activity Assay provides a simple, rate method for determining catalase activity in various biological and purified samples.

Test Principle:
Catalase enzyme activity can be measured by monitoring the consumption of H_2O_2 substrate at 240 nm.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$
(Absorbance at 240 nm ↓) (no Absorbance at 240 nm)

Unfortunately, H_2O_2 levels above 0.1 M cause rapid inactivation of catalase even though enzyme saturation requires up to 5M H_2O_2 substrate. For this reason, accurate measurement of catalase activity requires that substrate be present at fairly low concentration. Accordingly, one unit of catalase activity is classically defined as the amount of enzyme that will decompose 1.0 µMole H_2O_2 substrate (starting concentration = 10.3 mM) per minute at pH 7.0 and 25°C. The precise starting substrate requirement requires that a tedious calibration of H_2O_2 be performed prior to assay.

The NWLSS™ Catalase Activity Assay is based on the method of (Beers and Sizer (1952) with the following modifications to increase robustness and convenience.

1. Catalase calibrator of known activity is provided in order to negate the need for tedious H_2O_2 substrate calibrations.

2. Reagents have been formulated to provide better stability of ***Diluted H_2O_2 Substrate*** and ***Diluted Catalase Standards*** allowing for more convenient test conditions.

General Specifications:

Format:	Cuvette or 96 Well Microplate	
Number of Tests:	30 Cuvette or 96 Microplate	
Specificity:	Catalase Enzyme	
Sensitivity:	LLD =	0.3 U/mL in Reaction Mix
		6 U/mL in Sample Added to Reaction Mix

Kit Contents:

Sample Dilution Buffer:	1 X 30 mL bottle
Assay Buffer:	1 X 30 mL bottle
Hydrogen Peroxide (H_2O_2) Reagent:	1 Vial
Catalase Standard:	1 vials

Required Materials Not Provided:

Disposable semi-micro UV-cuvettes (1.0 mL), or UV microplate
Microcentrifuge tubes.
Plastic or glass bottles.
Pipettors, adjustable 0.0 – 1.0 mL (8-channel pipettor – 300 µL for microplate assay).
Disposable pipette tips.

Required Instrumentation:

Spectrophotometer (capable of recording UV absorbance at least every 2 seconds), or microplate reader (capable of recording UV absorbance in kinetic mode).

Warnings, Limitations, Precautions:

Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician

Storage Instructions:

Upon receipt, store the reagents at 2-8°C. Do not use components beyond the expiration date printed on the label.

Assay/Instrument Preparation:

Spectrophotometer (Cuvette Assay) Setup

Turn on spectrophotometer and let it warm up according to manufacturer's instruction for UV measurement (relatively constant room temperature lab environment is recommended).

Plate Reader (Microplate Assay) Setup

Turn on microplate reader and let it warm up according to manufacturer's instruction for UV measurement (relatively constant room temperature lab environment is recommended). Set wavelength to 240 nm, and set up microplate layout and other parameters.

Assay/Instrument Preparation (continued):

Note:

Because data must be recorded within ~30 seconds after mixing sample and assay cocktail, the assay can only be performed ***one column (8 wells) or one well at a time.***

Consult microplate reader manual for the minimum read interval in kinetic mode. The interval is typically listed for 96-well reading and 1/10 of the interval is needed to read a whole column of wells with approximately seven (7) data points needed to obtain the reaction rate. We do not recommend testing more than one microplate column at the same time.

Reagent Preparation:

Sample Dilution Buffer:

Supplied Ready to Use.

Working H_2O_2 Reagent:

When first removing from cold storage, add entire contents of H_2O_2 Reagent (approximately 460 µL) as supplied to Assay Buffer and label as **Working Assay Buffer.** Wash the vial three times with the mixed solution and combine to the **Working Assay Buffer** to assure full transfer of contents. After mixing, let stand for 2 hours before using. Catalase standards and samples should be assayed using this **Working Assay Buffer** at room temp. within 2.5 hours else reagent can be stored overnight at 4°C (warming up to room temperature again before using).

Catalase Standard:

Just before use, add 850 µL of Sample Dilution Buffer to the Catalase Standard vial, the resultant solution is 150 U/mL in catalase activity. Dilute further in additional four microcentrifuge tubes by mixing following:

Standard Number	Sample Dilution Buffer (µL)	150 U/mL Standard (µL)	Standard Concentration (U/mL)
1	300	0	0.0
2	225	75	37.5
3	150	150	75.0
4	75	225	112.5
5	0	300	150.0

Note:

The reconstituted Standard Solution should be used within 2 hours at room temperature. If desired, a portion of the standard may be immediately placed at 4°C. and used the next day however, **reconstituted Catalase Standard is not suitable for longer-term storage.** Do not freeze reconstituted standard as this will inactivate the enzyme. Total assay time (30 tube assays) is approximately 1.5 hours.

Sample Handling/Preparation:

The multi-disciplinary interest in measuring catalase enzyme activity has resulted in a myriad of sample types and experimental conditions and is beyond the scope of this product insert to describe sample processing in detail for each case. However, general guidelines are provided below for representative sample types.

Sample Stability

Catalase is reasonably stable at high concentration. However, relatively rapid decline in activity has been observed in diluted samples. For example, catalase in concentrated hemolysates (~5g Hb/mL) is stable for 6 days at 2-8°C, whereas activity at 1.2 mg Hb/mL has been shown to decrease by 10-15% in 24 hours. Freezing at –20°C should be strictly avoided even for concentrated samples. For example, RBC lysate stored at –20°C may lose 40% of catalase activity. If necessary, concentrated samples should be stored at –70°C or lower to avoid activity loss.

Sample Dilution:

It is important to let diluted samples stand for 15 minutes before assaying. **When diluted according to instructions, the Sample Dilution Buffer formulation in the NWLSS™ Catalase Activity Assay helps stabilize both sample and standard catalase after dilution up to 2 hours at room temperature.** Total assay time for 30 tube assays is approximately 1.5 hours.

Diluted samples can be placed on ice and be assayed simultaneously in high-throughput microplate format to ensure accurate results.

Suggested Dilutions:

RBC Lysate (RBC:dH_2O at 1:4)	1/100X
Tissues Homogenates (5% or 10% with 0.01% digitonin or 0.25% sodium cholate detergent)	1/20X-1/100x

If the reaction rate exceeds the highest catalase standard rate, it is important not to use the linear regression equation of the standard curve to calculate catalase activity in the sample. Rather, adjust the dilution factor, make a new dilution and re-assay the sample.

Assay Protocol:

Cuvette Assay:

1. To a clean cuvette, add 1000 µL of **Sample Dilution Buffer**. Place it in the reference cuvette holder. Set wavelength to 240 nm and zero the instrument.
2. To a clean semi-micro UV cuvette, add 950 µL of **Working Assay Buffer**
3. Pipette 50 µL of diluted standard or sample to the cuvette, mix as quickly as possible by repeated pipetting (~10 times) with the same pipette tip, or by capping/inverting the cuvette.
4. Immediately start recording the absorbance at 240 nm, every 2 second or at the smallest time interval allowed for 0.25 minutes (15 seconds).

Note:
Because data must be recorded within 30 seconds, do not allow pipetting/mixing time to exceed 15 seconds. Keep the same pace of pipetting/mixing throughout the whole experiment. A practice run with a timer using Sample Dilution Buffer or dH_2O is recommended.

Microplate Assay:

See note about maximum number of wells (8) on page 5 of this insert under **Assay/Instrument Preparation** section.

1. To each clean UV microplate well, pipette 15 µL of diluted standard or sample.
2. Add 290 µL of Assay Cocktail to each well . Use an 8-channel pipettor if assaying 8 wells simultaneously. Mix as quickly as possible using reader's shaker function.
3. Immediately start recording absorbance at 240 nm, every 2 second or at the smallest time interval allowed for 0.25 minutes (15 seconds).

Note:
Because data must be recorded within 30 seconds, do not allow pipetting/mixing time to exceed 15 seconds. Keep the same pace of pipetting/mixing throughout the whole experiment. A practice run with a timer using Sample Dilution Buffer or dH_2O is recommended.

Data Analysis:

The decomposition rate of hydrogen peroxide follows a first order reaction with H_2O_2 concentration, and is linear for the first half minute (30 seconds) of reaction (see Figure 1).

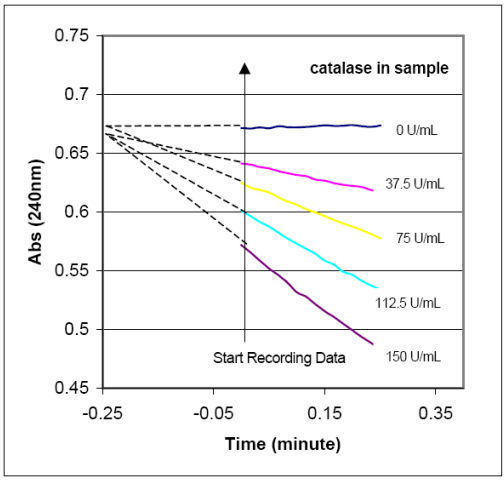


Figure 1: Rate curves of hydrogen peroxide decomposition catalyzed by bovine liver catalase. Catalase unit represents concentration in sample added, not in final assay mixture. 50 µL of sample was added to 950 µL of assay cocktail, addition and mixing took about 0.25 minutes. Data recording started at time 0 minute. The experiment was performed at room temperature without a constant temperature curvette holder.

The decomposition rate increases with the presence of higher concentration of catalase. Without complete mixing, it is not possible to record data when sample and reaction cocktail are initially mixed. Complete mixing in a cuvette typically requires about 10 seconds with repeated pipetting. After mixing, the reaction proceeds at a rapid rate with only ~15 seconds available to record the decomposition rate data. Typical rate curves of standards ranging from 0 U/mL to 150 U/mL are shown above in Figure 1. The rate of reaction can be obtained by linear regression. In the regression equation:

$$Y = -aX + b,$$

where a is the decomposition rate or (ΔAbs_{240nm}/min).

A less preferred way to obtain the rate is to select two data points in the rate curve and to calculate rate according to following equation:

$$\text{Rate } (\Delta Abs_{240nm}/\text{min}) = -(Y_2 - Y_1)/(X_2 - X_1).$$

The decomposition rates obtained for the Diluted Standards are used to plot a standard curve (rate vs catalase concentration, see Figure 2). against which samples are then compared.

Data Analysis (cont.):

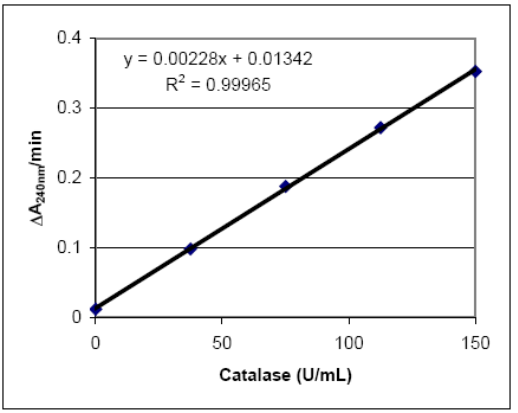


Figure 2: Standard curve obtained from linear regression rates of hydrogen peroxide decomposition in the presence of catalase.

The linearity of standard curve is lost when catalase concentration exceeds 180 U/mL due to rapid consumption of hydrogen peroxide (data not shown). Therefore, if a sample yields a decomposition rate higher than that of the 150 U/mL catalase standard, the sample should be additionally diluted and assayed again.

Calculations

1. Plot the standard curve as illustrated in Figure 2. Obtain the slope (a) and intercept (b) of the curve by regression:

$$Y = aX + b$$

or

$$\text{Rate} = \text{Slope}[\text{Catalase}_{ds}] + \text{Intercept}$$

2. Rewriting and rearranging the equation, the catalase concentration of diluted samples [Catalase_{ds}] added to the reaction mixture can be calculated as:

$$[\text{Catalase}_{ds}] = (\text{Rate} - \text{Intercept})/\text{Slope}$$

where Rate is the decomposition rate (ΔA_{240nm}/min) of the sample.

3. Catalase concentration of the original sample [Catalase_{os}] is calculated by multiplying the diluted sample result by the dilution factor:

$$[\text{Catalase}_{os}] = [\text{Catalase}_{ds}] * df$$

Performance Details:

Stability	All unopened reagents are stable until the expiration date stated on the package label when stored at 2-8°C.
Sensitivity:	The lower limit of detection is 0.3 U Catalase/mL reaction mixture or, 6.0 U Catalase/mL diluted sample added to reaction mix.
Dynamic Range:	0.3—150 U/mL in the assay.
Linearity:	Excellent linearity is maintained up to 150U catalase/mL sample.
Assay Precision:	The intra-assay coefficient of variation is 7% for the 37.5 U/mL Catalase Standard.
Recovery:	The recovery for spiked catalase in RBC lysate was found to be 110% using this assay.

References:

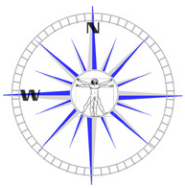
1. Beers, R. F. Jr. and Sizer, I. W., "A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase" *J. Biol. Chem. 195*, 133-140 (1952).

2. Aebi, H., "Catalase *in Vitro*" *Methods in Enzymology* 105, 121-126 (1984).

Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

End User Notes:



Northwest
Life Science Specialties, LLC

5131 NE 94th Avenue, Suite 201
Vancouver, WA 98662
Phone 360-449-3091 or Toll Free: 888-449-3091
Fax 360-449-3092
E-mail: sales@nwlifescience.com