

**Northwest**  
Life Science Specialties, LLC

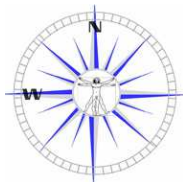
Premier Products for Superior Life Science Research

*NWLSSTM*  
*Catalase Activity Assay*

Colorimetric Assay for Catalase Enzyme Activity

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Product NWK-CAT02  
*For Research Use Only*



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Simple assay kit for quantitative measurement of catalase enzyme activity in biological samples such as tissue homogenates & cell lysates.

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**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.

**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.

Catalase enzyme activity can be measured by monitoring the consumption of  $H_2O_2$  substrate 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  $H_2O_2$  substrate be present at fairly low concentration and one unit of catalase activity is classically defined as the amount of enzyme that will decompose 1.0  $\mu$ Mole  $H_2O_2$  substrate (starting concentration = 10.3 mM) per minute at pH 7.0 and 25 °C. This precise substrate requirement requires that a tedious calibration of  $H_2O_2$  be performed and the requirement of kinetic measurement does not lend itself to high-throughput assay.

**Intended Use:**

The colorimetric NWLSS™ Catalase Activity Assay is intended as a simpler, non-UV method for the quantification of catalase enzyme activity in biological samples such as plasma, tissue homogenates or cell lysates. The assay is not species specific and is compatible in all model systems where catalase enzyme is thought to be present.

**Test Principle:**

The colorimetric NWLSS™ Catalase Activity Assay contains an assay cocktail containing  $H_2O_2$  that is incubated with catalase containing samples for exactly 2 minutes. The catalase reaction is quenched by adding an inhibitor. Remaining  $H_2O_2$  is measured by Horseradish Peroxidase (HRP) catalyzed oxidation of a chromogen to generate a blue colored cation free radical with  $\lambda_{max}=653$  nm. Sample catalase concentration is determined by comparing sample absorbance at 653 nm (650 nm is OK) to that of a certified catalase standard, eliminating the need to calibrate precise  $H_2O_2$  concentration as with other assays. Experiments can be carried out at room temperature under conditions that are more accurate and convenient. Modifications are also made in our formulations to overcome problems associated with instability of diluted enzyme standards at the room temperature. The assay principal is summarized in the reaction schemes on the following page:

**Test Principle (continued):**

Allow the reaction (1) to proceed for precisely 2 minutes, add stop solution. Use a small portion of the reaction (1) mixture in reaction (2).



The HRP-catalyzed reaction is optimized with respect to the amount of  $\text{H}_2\text{O}_2$  used in the catalase reaction. This yields a linear response between the absorbance at 650 nm and the amount of  $\text{H}_2\text{O}_2$  remaining creating an inverse linear correlation with the amount of catalase activity.

**General Specifications:**

Format: 96 well microplate or 30 Cuvette assays  
 Specificity: Catalase Enzyme Activity  
 Sensitivity: LLD = 10U/mL in sample assayed

**Kit Contents:****Materials provided for 1 X 96 microplate or 30 test tube tests:**

- 4X Assay Buffer 30 mL
- $\text{H}_2\text{O}_2$  Solution 1 Vial
- Sample Dilution Buffer 30 mL
- Catalase Standard (150 U) 1 Vial
- 20X Stop Solution 1.5 mL
- Chromogen Solution (TMB in DMSO) 1.8 mL
- Horse Radish Peroxidase 1 Vial

**Required Materials Not Provided:**

Spectrophotometer, or microplate reader.  
 Disposable microplate or semi-micro cuvettes (1.0 mL)  
 Microcentrifuge tubes (1.0 mL – 1.8 mL).  
 Plastic bottle (3x30 mL)  
 Pipettors, adjustable from 0.0 – 1.0 mL  
 8-channel or 12-channel pipettors – 20  $\mu\text{L}$  and 300  $\mu\text{L}$  for microplate assay.  
 Disposable pipette tips.

**Required Instrumentation:**

Microplate reader with 450nm and 650 nm capability.

**Data Analysis (continued)**

1. Obtain the slope (a) and interception (b) of the linear correlation curve by regression:

$$y = ax + b$$

2. The catalase concentration in the sample added to the reaction mixture (not concentration in the reaction mixture) is:

$$C_i = (A_i - b)/a$$

where  $A_i$  is the measured absorbance value at 650 nm of sample i.

3. The catalase concentration in the original sample is:

$$C^0 = C_i * (\text{Dilution Factor})$$

**Assay Performance:**

*Lower limit of detection (LLD):* 10 U catalase/mL sample

*Linearity:* Linearity is maintained up to 100 U catalase/mL sample

*Precision:* Intra-assay coefficient of variation 8.5% at 50 U/mL

*Recovery:* 92% when spiked in RBC lysate

**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).

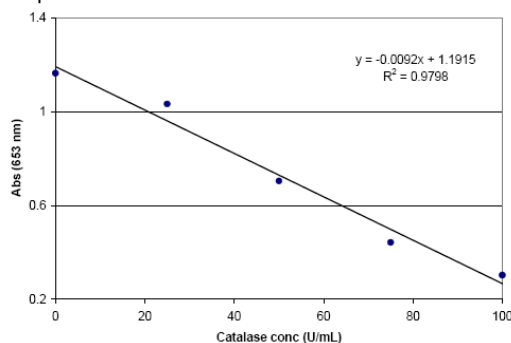
**Assay Protocol:**

1. Add 10  $\mu\text{L}$  of **sample** or **standard** to the bottom of a micro-centrifuge tube or deep well microplate.
2. Pipette 190  $\mu\text{L}$  of **Working Assay Cocktail** into each tube or microplate well...mix quickly by capping/inverting or by repeated pipetting.
3. Let the solution incubate for exactly 2 minutes.
4. Add 200  $\mu\text{L}$  of **Stop Solution** in the same manner and order used in Step 2...mix quickly. This reaction mix will be used in Step 5C.
5. *For cuvette assay:*
  - A) To each semi-micro cuvette, add 880  $\mu\text{L}$  of **Working Assay Buffer**.
  - B) Add 50  $\mu\text{L}$  of **Chromogen Solution**.
  - C) Add 20  $\mu\text{L}$  of reaction mixture from step 4.
  - D) Add 50  $\mu\text{L}$  of **Working HRP solution**.
  - E) Mix quickly then incubate for 7 minutes.
  - F) Immediately measure absorbance at 650 nm using a cuvette spectrophotometer.
5. *For microplate assay:*
  - A) Add 264  $\mu\text{L}$  of **Working Assay Buffer** to each well used. Use multichannel pipettors for faster through-put.
  - B) Add 15  $\mu\text{L}$  of **Chromogen Solution**.
  - C) Add 6  $\mu\text{L}$  of reaction mixture from step 4.
  - D) Add 15  $\mu\text{L}$  of **Working HRP solution**.
  - E) Mix quickly then incubate for 7 minutes.
  - F) Immediately measure absorbance at 650 nm using a microplate reader.

**Data Analysis:**

Create a standard curve by plotting Absorbance vs. Concentration for each standard level assayed. If available, set the plate reader to utilize linear curve fit. An example standard curve is shown below.

Figure 1: Standard curve of bovine liver catalase. Catalase unit represents concentration in sample added, not in final assay mixture. The experiment was performed at room temperature of 25 °C. Also shown is a linear regression equation between absorbance at 653 nm and catalase concentration.

**Warnings, Limitations, Precautions:**

Let kit warm up to room temperature (~ 2 hours) before using.

The reconstituted Standard Solution should be used within 2 hours at room temperature and is not suitable for longer-term storage. If portions of standards are saved immediately, they can be used again after overnight storage at 4 °C.

**Storage Instructions:**

The kit should be stored complete in the original box in a refrigerator at 4 °C. Stored in this manner the kit is stable until the expiration date printed on the product label.

**Instrument Preparation:****Plate Reader Setup**

Wavelength: 650 nm

Mode: Endpoint

Shaker: On

Suggested Curve Fit: Linear

**Reagent Preparation:**

Notes before using:

1. Let kit warm up to room temperature (~ 2 hours) before using.
2. Undiluted **4X Assay Buffer** is used in preparation of...
  - Working Assay Cocktail**
  - Working Stop Solution**
  - Working Assay Buffer**
 Working Assay Buffer is only used in preparation of...
  - Working HRP Solution**
  - Working Catalase Standard**
3. Working Assay Cocktail can be made during warm up period.

**Reagent Preparation (continued):**

**Assay Cocktail:** (Use 4X Assay Buffer, dH<sub>2</sub>O & H<sub>2</sub>O<sub>2</sub> Solution)  
To a clean plastic bottle, add 5.0 mL of **4x Assay Buffer**, then add 14.9 mL dH<sub>2</sub>O and add 100.0 µL of Hydrogen Peroxide. Mix well. Label as **"Working Assay Cocktail"** and incubate 1 hour before using. Working Assay Cocktail is stable for at least 2.5 hours at room temperature, and can be stored overnight at 4 °C. Warm up to room temperature again before using.

**Assay Buffer:** (Dilute 4X Assay Buffer with dH<sub>2</sub>O)  
To a clean plastic bottle, add 7.5 mL of **4x Assay Buffer**, add 22.5 mL dH<sub>2</sub>O. Mix and label as **"Working Assay Buffer"**.

**Stop Solution** (Dilute with 4X Assay Buffer)  
To a clean plastic bottle, add 5.0 mL of **4x Assay Buffer**, 14.0 mL of **dH<sub>2</sub>O**, then add 1.0 mL of **20x Stop Solution**. Mix.

**Horseradish Peroxidase** (Dilute with Working Assay Buffer)  
Add 1.8 mL of **Working Assay Buffer** to the HRP vial. Shake briefly to dissolve. Label as **"Working HRP Solution"**. This solution is stable for 4 hours at room temperature. A portion can be stored overnight at 4 °C. Warm up to room temperature again before using.

**Chromogen Solution:** (Supplied Ready To Use)  
Chromogen solution is stored frozen at 4 °C. If it is not totally thawed, hold in hand and shake briefly. Unused portion can be stored at 4 °C overnight. It can be used again after warming up.

**Catalase Standard:** (Dilute with Sample Dilution Buffer)  
**Warning: Reconstituted standards are only stable for 2 hours at room temperature. Do not reconstitute and make subsequent dilutions of standard until just before use. Standard can be stored overnight at 4 °C if refrigerated immediately after reconstitution. Standards should not be used after 24 hours. Do not freeze reconstituted standards due to enzyme inactivation upon freezing.**

Add 1.5 mL of **Sample Dilution Buffer** to the **Catalase Standard** vial, the resultant solution is 100 U/mL in catalase activity. Dilute additionally in four microcentrifuge tubes according to the following table:

Standard No.	Sample Dilution Buffer (µL)	100 U/mL Standard (µL)	Standard Conc. (U/mL)
1	400	0	0.0
2	300	100	25.0
3	200	200	50.0
4	100	300	75.0
5	0	400	100.0

**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 Storage:**  
**Store concentrated samples at -70 °C or lower temperature to avoid activity loss.**

Freezing at -20 °C is to be avoided even for concentrated samples... RBC lysate stored at -20 °C shows a 40% loss in activity.

**Sample Processing:**  
Catalase is reasonably stable at high concentration... Catalase activity in concentrated hemolysates (~5g Hb/mL) is stable for 6 days at 4 °C.

Catalase activity can decline rapidly in diluted samples... Catalase activity decreases by 10-15% in 24 hours at ~1.2 mg Hb/mL.

The **Sample Dilution Buffer** formulation in the NWLSS™ kit stabilizes catalase activity after dilution up to 2 hours at room temperature. It is important to incubate diluted samples for 15 minutes before assaying. Diluted samples can be placed on ice for assaying simultaneously in high throughput microplate format to ensure accurate results.

Following are approximate dilution guidelines using the Sample Dilution Buffer included in this kit:  
**\*Note: All samples must be incubated 15 minutes after dilution before assaying.**

RBC lysate (1:4 RBC:dH<sub>2</sub>O): 1/100x

Tissue Homogenate (5% - 10% with 0.01% digitonin or 0.25% sodium cholate detergent): 1/20x - 1/100x

**\*\*Note: If absorbance reading at 650 nm is below that of the highest catalase standard, 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 and make a new dilution.**