Product Manual

Catalase fluorometric detection Kit

Instruction Manual ADI-907-027

Sufficient Reagents for 500 tests

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INTRODUCTION

The Catalase fluorometric detection kit is a sensitive fluorescent assay to detect catalase activity by measuring the amount of substrate (hydrogen peroxide) remaining after sample addition. This kit provides a simple homogenous assay that is adaptable to kinetic and high throughput applications. Catalase is an antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H_20_2) to water and oxygen. Catalase is ubiquitously expressed in mammalian and non-mammalian aerobic cells containing the cytochrome system and is a protein tetramer of four ferrihemoprotein groups per molecule. The enzyme has been isolated from various sources, including bacteria and plant cells[1-3]. Catalase activity varies greatly from tissue to tissue. Highest activity is seen in liver and kidney, while lowest activity is seen in connective tissue[3]. The presence of catalase in the peroxisomes of mammalian cells helps to alleviate oxidative damage by catalyzing the metabolism of the peroxide substrate[4]. The production of H_20_2, a highly reactive byproduct of mammalian metabolism, can be deleterious to cells and is an end product result of various oxidase and superoxide dismutase reactions. Accumulation of H_20_2 can result in cellular damage through oxidation of proteins, DNA, and lipids thus resulting in cell death and mutagenisis[8-11]. The role of H_20_2 in oxidative stress related pathologies (e.g., inflammation, cancer, diabetes, cardiovascular disease, anemia, Parkinson's disease, Alzheimer's disease) has been widely studied[8, 12].

PRECAUTIONS

The Catalase Fluorometric Detection Kit is a sensitive assay that utilizes a non- fluorescent detection reagent to measure H_20_2 substrate left over from the catalase reaction[5-6].

Reaction:

H2O2 + Catalase ⟶ Water + Oxygen

↓

H202 (left over) + Detection reagent (non-fluorescent) + Peroxidase

----------> Resorufin (fluorescent)

Excitation 530-571 nm

Emission 590-600 nm

MATERIALS PROVIDED

Catalase Reaction Buffer Concentrate

20 mL, Product No. 80-1697 A 5X Buffer Concentrate

Detection Reagent

1 vial, Product No. 80-1698

One vial is sufficient for 500 tests

Horseradish Peroxidase Concentrate

1 vial, Product No. 80-1699

18.9 units of enzyme

Hydrogen Peroxide

200 µL, Product No. 80-1700

A 3% stabilized solution of hydrogen peroxide

Catalase Enzyme

1 vial, Product No. 80-1701

A crystalline suspension of active catalase enzyme

STORAGE

All components of this kit are stable at 4°C.

OTHER MATERIALS NEEDED BUT NOT SUPPLIED

- Dimethyl sulfoxide (DMSO)
- Black 96 well plates
- Fluorescence plate reader
- Deionized water
- Superoxide dismutase (optional, see Reagent Preparation #3)

REAGENT PREPARATION

1. 1X Reaction Buffer

Prepare a 1X solution of the Reaction Buffer by diluting 4 mL of Reaction Buffer Concentrate with 16 mL of deionized water. This can be stored at 4ºC until the kit expiration date, or for 3 months, whichever is earlier.

2. 100X HRP

Prepare a 40 Units/mL (100x) solution of Horseradish Peroxidase (HRP) by diluting it with 1X Reaction Buffer. See the vial label for the protein concentration and units per mg of activity. For example, if the protein concentration is 6.5 mg protein/mL and specific activity is 386 U/mg of protein, then the enzyme concentration is 6.5 X 386 = 2509 Units/mL. To prepare 500 µL of a 40 U/mL concentration, add 8 µL of undiluted HRP to 492 µL of 1X Reaction Buffer. Make enough HRP for a days' worth of experiments.

3. Detection reagent

Dissolve the contents of one vial in 500 µL DMS0. Allow the contents to sit at room temperature for 15 minutes. Gently pipet up and down several times to dissolve any clumps. 0nce dissolved the detection reagent should be used promptly and any remaining reagent can be aliquotted and stored at -70ºC. Avoid repeated freeze thaw cycles.

At NADH levels above 10µM and Glutathione levels above 300 µM, detection reagent oxidation results from side chain reaction between NADH and HRP. This could result in aberrant readings. To minimize this interference it is recommended to add superoxide dismutase (S0D) at 40 U/mL to the reaction[7].

4. Hydrogen Peroxide (40 µM)

The 3% hydrogen peroxide solution provided is ~ 0.881 M. Prepare a 40 µM solution of the hydrogen peroxide in 1X Reaction Buffer. This will serve as the substrate for catalase.

5. Catalase Standard Curve

See vial for specific catalase activity and concentration. The Catalase is a crystalline suspension in water. This crystalline suspension must be dissolved before use. Vortex the vial to evenly disperse the crystalline suspension. Pipet an appropriate amount of catalase into 1X Reaction Buffer. Warming gently at 30ºC and slight agitation will help dissolve the catalase crystals.

One unit will decompose 1.0 µmole of H_2O_2 per min at pH 7.0 at 25°C,

while the H_2O_2 concentration falls from 10.3 to 9.2 mM, measured by the rate of decrease of A_{240}.

Prepare a standard curve by diluting the appropriate amount of Catalase in 1X Reaction Buffer. A suggested standard curve range is 0 to 4 units/mL.

6. Reaction Cocktail

Prepare 10 mL **Reaction Cocktail** for 100 assays:

100 µL Detection Reagent

100 µL 100X HRP.

9.8 mL of 1X Reaction buffer

The volume of the **Reaction Cocktail** can be scaled down or up (as needed) provided that the ratios of the ingredients are kept constant.

The fluorescent product of the detection reagent is not stable in the presence of thiols (e.g. DTT or 2-mercaptoethanol). Keep these reactants below 5 µM. If using your own buffer, keep the reaction between pH 7-8 (pH 7.4 is optimal).

SAMPLE HANDLING

Dilute samples in 1X Reaction Buffer. If the general amount of catalase activity is not known, make several dilutions (1, 10, 20, 50 fold dilution) of your sample. 0ne of these dilutions should fall in the standard curve range.

ASSAY PROCEDURE

1. Pipet 50 µL of standard or sample to the bottom of a 96 well black plate.
2. Pipet 50 µL of the 40 µM H_2O_2 solution to each well.
3. Incubate the plate for 30 to 60 minutes at room temperature.
4. Add 100 µL of the Reaction Cocktail to each well.

Note: Each investigator should optimize incubation times for their particular application.

5. Incubate the plate for 10 to 15 minutes.
6. Read samples using excitation 530-570 nm (570 nm is optimal) and measure fluorescence at 590-600 nm.

Calculation of Results

Several options are available for the calculation of Catalase activity in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the Catalase activity can be calculated as follows:

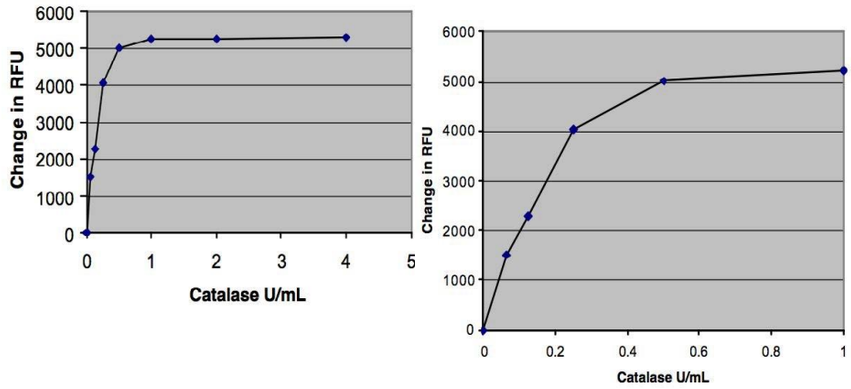
Calculate the average net RFU for each standard and sample by subtracting the average 0 U/mL RFU from the average RFU for each standard and sample.

Plot the Average Net RFU for each standard versus Catalase Activity (U/mL) in each standard. Approximate a line through the points. The concentration of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be reanalyzed using a higher dilution.

Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay. The reaction contained 20 µM H_20_2 (final) per well and the indicated amounts of catalase in 1X reaction Buffer. The reaction was incubated for 30 minutes at room temperature. The graph reports the change in fluorescence, observed fluorescence from negative control (no catalase) minus catalase sample fluorescence.



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NOTES

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