interchim

Fluoro: Catalase™

Fluorescent Catalase Detection Kit

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Updated – 1/07

CELL TECHNOLOGY

I. Assay Principle:

Catalase is an antioxidant enzyme that catalyses 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. 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). In eukarotic cells, catalase in concentrated in organelles called peroxisomes (4).

The production of hydrogen peroxide in eukaryotic cells is an end product result of various oxidases 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). H_20_2 role in oxidative stress related diseases have been widely studied (8,12).

The Fluoro Catalase detection kit is a sensitive assay that utilizes a non- fluorescent detection reagent to measure H_2O_2 substrate left over from the catalase reaction (5-6).

Reaction:

H_2O_2 + catalase → Water + oxygen.

H_2O_2 (left over) + Detection reagent (non-fluorescent) + Peroxidase → Resorufin(fluorescent)
Excitation 530-571nm
Emission 590-600nm.

II. Storage:

The kit contains multiple storage temperature components. Please see labels of individual components for storage instructions.
Once a vial of the Detection reagent is opened, it should be used promptly and frozen since it is subject to oxidation by air.

III. Warnings and Precautions:

1. For Research use only. Not for use in diagnostic procedures.
2. Practice safe laboratory procedures by wearing protective clothing and eyewear.
3. The fluorescent product of the detection reagent is no stable in the presence of thiols (DTT or 2-mercaptoethanol). Keep these reactants below 5μM.

IV. Part # 5019. Kit contents and Storage (for 500 assays).

Unopened kit can be stored at 4-8^{0}C for 1 week. Refer to individual components for storage conditions

Description	Part#	Storage after opening Kit or Reconstitution of Reagents.
1 Bottle: 5X Reaction Buffer pH 7.4	3021	4-8^{0}C
1 vial: Detection reagent	4010	Aliquot in single use vials: Below –20^{0}C
1 vial: Horseradish Peroxidase	6009	4-8°C undiluted. Diluted: below -20°C
1 vial: Hydrogen Peroxide	3022	4-8^{0}C.
1 vial: Catalase Enzyme	6008	Crystalline suspension: 4-8^{0}C

Materials required but not supplied:

1. Dimethyl sulfoxide (DMSO)
2. Black 96-well plates
3. Fluorescence plate reader
4. Deionized water

V. Preparation of reagent working solutions:

1. **1 X Reaction Buffer Part# 3021:** To prepare a 1X solution of Reaction buffer, dilute the 5X buffer 1:5 with Di water. For example to prepare 20 ml of 1X Reaction buffer add 4ml of 5X Reaction buffer to 16ml of deionized water.

2. **Horseradish Peroxidase Part# 6009:** Make a 40 Units/mL (100X) stock solution of 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 6.5mg 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 500µL of a 40U/mL concentration, add 8µL of undiluted HRP to 492µL of 1X Reaction Buffer. Make enough HRP for a days' worth of experiments.

   This 100X stock maybe stored for several weeks at or below -20^0C. Aliquot in single use vials.

   Note: If Part # 6009 does not mention protein concentration and units per mg on the label, use the following values: 13mg/mL at 291 units/mg.

3. **Detection Reagent Part# 4010:** Dissolve the contents of one vial in 500µL of DMSO. Allow the contents to sit at Room Temperature for 15 minutes. Next gently pipette up and down several times to dissolve any clumps. Once dissolved the detection reagent should be used promptly and any remaining reagent can be aliquated and frozen at -70°C. Avoid repeated freeze thaw cycles.
   Note: Protect the Detection Reagent from light.

4. **Hydrogen Peroxide 3%. Part# 3022:** The hydrogen peroxide is at 0.881M (3%). Make a 40µM dilution of the hydrogen peroxide in 1X reaction buffer. This will serve as a substrate for catalase.

5. **Catalase Part# 6008**. See vial for specific catalase activity and concentration. The Catalase is a crystalline suspension in water. This crystalline suspension needs to be dissolved before use. Gently centrifuge the vial (material maybe trapped on the cap) and then vortex the vial to evenly disperse the crystalline suspension. Pipette up and down several times before pipetting appropriate amount of catalase into 1X reaction buffer. Warming (30^{0}C) 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 conc. falls from 10.3 to 9.2 mM, measured by the rate of decrease of A_{240}).

VI. Assay Protocol: Detection of catalase activity in Samples.

1. Prepare 10ml **reaction cocktail** for 100 assays:
   100µL Detection Reagent .
   100µLl HRP of 100X HRP.
   9.8ml 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.
   Note: *See Technical note #1.*

2. Standard Curve: Prepare a standard curve of catalase by diluting the appropriate amount of catalase (from step V 5 above) in 1X reaction buffer. A suggested standard curve range is 0-4 U/mL.

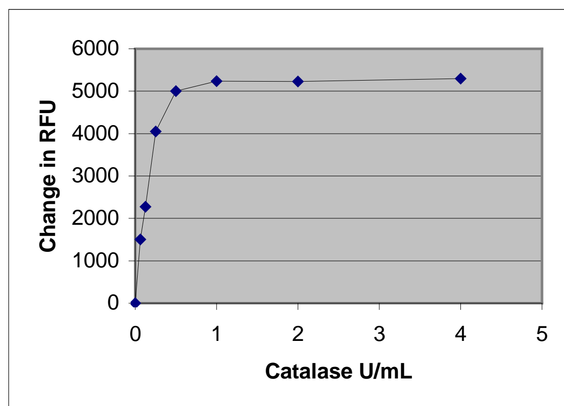
3. Preparation of samples.
   Dilute the catalase containing samples in 1X reaction buffer. If the general amount of catalase activity is not known, make several dilutions (1, 10, 20, 50 fold dilution) of your sample. One of these dilutions will fall into the standard curve range.

4. Assay.

   1. To a black 96 well plate add 50 µL of sample or standard curve, to each individual well. Next add 50 µL of hydrogen peroxide, from step V #4, to each well.
   2. Incubate plate 30 –60 minutes at room temperature.
   3. After the incubation time add 100 µL of Reaction Cocktail prepared in Section VI step 1 above.

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

   4. Incubate 10 –15 minutes.
   5. Read samples using excitation: 530-570nm (570nm is optimal) and measure fluorescence at 590-600nm.



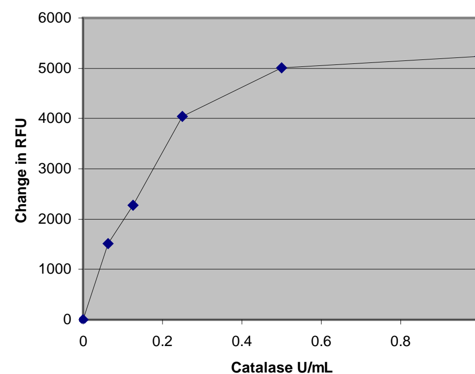


Figure 1. Catalase activity was detected using the Fluoro Catalase kit. The reaction contained 20uM H_2O_2 (final) per well and the indicated amounts of catalase in 1X reaction Buffer. The reaction was incubated for 30 minutes at room temperature. Next 100uL of Reaction cocktail was added to each well and the reaction incubated for another 10 minutes in the dark at room temperature. Fluorescence was measured at excitation 530nm and emission detected at 590nm. The graph reports the change in fluorescence, observed fluorescence from negative control (no catalase) minus catalase sample fluorescence.

VIII. Technical Notes:

1. The fluorescent product of the detection reagent is no stable in the presence of thiols (DTT or 2-mercaptoethanol). Keep these reactants below 5μM.
   If you are using your own buffer, keep the reaction between pH 7-8 (optimal pH 7.4).

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

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