

## Application Note EuTc 003:

# One-step Kinetic Detection of Catalase by EuTc Reagent

### Introduction

Catalase (hydrogen peroxide : hydrogen peroxide oxidoreductase, EC 1.11.1.6), is capable of decomposing hydrogen peroxide (HP) without producing free radicals<sup>(1)</sup>. It has long been recognized as a defense for the oxidative stress and plays an important role in the compartmentalization of HP in biological systems.

Catalase (CAT) is relatively unique compared to other members of the peroxidase family since it displays both catalase activity (EC 1.11.1.6; see reaction equation 1) and peroxidase activity (EC 1.11.1.7; equation 2). Typical substrates (SH<sub>2</sub> in reaction 2) include ethanol, methanol, formate, nitrite, and phenol.



Here, a novel fluorescent substrate for qualitative and quantitative determination of CAT is introduced, along with a new one-step kinetic fluorescent assay of CAT. The detection is based on the rather unexpected finding that the HP in the EuTc-HP system can be considered as a substrate by CAT, thus leading to a large decrease in its fluorescence.

### Experimental Setup

#### Materials

The following solutions should be used

*Solution A* (EuTc standard solution): dissolve the contents of Chromeon's EuTc vial in 100 mL of distilled water.

*Solution B* (5 mM hydrogen peroxide): dissolve 1 mL of 30% H<sub>2</sub>O<sub>2</sub> in 10 mL of distilled water to obtain a stock solution (stable for 4 weeks in a refrigerator). Dilute 50 µL of this stock to 10 mL with distilled water to obtain solution *B*. This solution should be prepared fresh daily.

*Solution C* (10 mM MOPS buffer): dissolve 2.3 g of MOPS sodium salt in 800 mL of distilled water, add 1.0 M HCl to adjust the pH to 6.9, and make up the volume to 1000 mL with distilled water.

*Solution D*: dissolve 100 µl of catalase (activity: ~51,100 Sigma units per mg, 25 mg /ml) in 10 mL of MOPS buffer (solution *C*); the solution may be further diluted to the concentration required.

#### Recommended Protocol:

In each well of a thermostated (30°C) 96-well microtiter plate, 20-65 µl of EuTc solution A, 20 µl of solution B, and MOPS solution C were added to get a volume of 250 µl. After 10 min, the samples containing catalase were finally added simultaneously into the wells, and the plates were under intervals of shaking and stabilization programmed by the reader software. Control experiments were conducted without adding catalase under similar conditions.

The kinetic change of the fluorescence was used for the quantification of the activity of catalase. The changes of fluorescence between 0 and 600 s were used for the calibration, versus the activity detected by UV spectrophotometric detection at 240 nm.

## Results

The results are summarized in the two figures below:

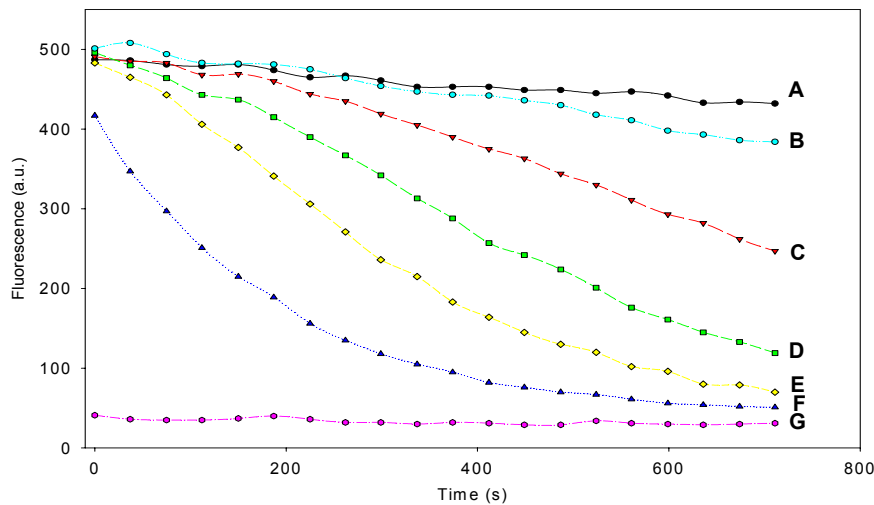


Figure 1. Kinetic determination of catalase using the reagent EuTc. (A), blank; (B) to (F), increasing activities of catalase; (G), no catalase, no H<sub>2</sub>O<sub>2</sub>.

Figure 1 shows typical graphs as obtained in the experiments described above. For the blank (A), the fluorescence shows no significant change during the time course. With increasing activity of catalase from (B) to (F), the fluorescence decreases as the results of HP consumption by catalase, while (G) is a contrast of EuTc alone without HP and catalase.

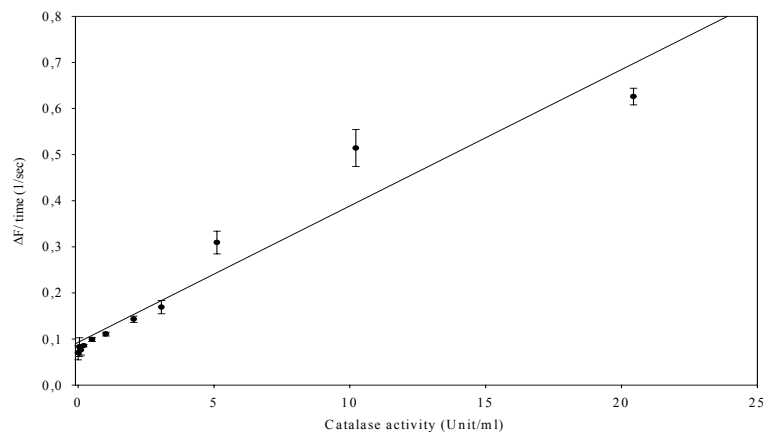


Figure 2. Typical calibration graph for catalase

A typical calibration curve for catalase is presented in Figure 2. The detection has a linear range between 1.6–20 unit/ml ( $r = 0.97$ ), with limit of detection at 1.6 unit/ml ( $S/N = 3$ ).

## Conclusion

The experiments above indicate that the method described here for CAT detection is a one-step and kinetic assay, at a clearly defined H<sub>2</sub>O<sub>2</sub> concentration, working at neutral pH, and without the need for adding other substrates. The assay can be performed successfully using microtiter-plates.

## Reference

1. Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) (1999) Enzyme Supplement 5. *Eur. J. Biochem.* 264, 610-650