

## Application Note EuTc 002:

# Hydrogen Peroxide Assay Using the EuTc Reagent

**Scheme:** EuTc (weakly fluorescent) + H<sub>2</sub>O<sub>2</sub> → EuTc-HP (strongly fluorescent)

### The following solutions should be used:

*Solution A* (EuTc working solution): dissolve the contents of Chromeon's EuTc vial in 100 mL of distilled water. Its absorbance at 405 nm is ~ 0.76 per cm.

### *Solution B*

(400 mM hydrogen peroxide): dissolve 1 mL of 30% H<sub>2</sub>O<sub>2</sub> hydrogen peroxide (HP) in 10 mL of distilled water to obtain a stock solution (stability: 4 weeks at 8°C). Dilute 50 µL of this stock by adding up to 100 mL with distilled water to obtain solution *B*. This solution should be prepared freshly each day.

### Recommended Protocol for Determination of Hydrogen Peroxide (HP) in Solution:

#### *Calibration Graph:*

This is established by mixing distilled water and solution *B* in a ratio of 1+9, 3+7, 5+5, 8+2, and 10+0 in a 10mL volumetric flask and thermostating at 25 °C (other temperatures also possible). Distribute 1 mL each of the diluted solutions and 1 mL of solution *A* into a cuvette thermostatted to the same temperature. Also pipet 1 mL of distilled water and 1 mL of reagent *A* into a cuvette to obtain a blank measurement. Measure, after 10 min, luminescence intensity at an excitation wavelength of 395 – 405 nm and an emission wavelength of 615 nm (at 8 – 10 nm bandwidth). Plot fluorescence intensity (or relative increase in fluorescence intensity) versus the concentration of HP.

*Cuvette Assay:* Place 1 mL of solution *A* in a thermostatted cuvette, add 1 mL of the aqueous sample containing 0.2 – 10 mg /mL<sup>-1</sup> of hydrogen peroxide, Measure fluorescence as given under *Calibration Graph*.

*Microplate Assay.* The assay may as well be performed in a microtiterplate format (96-wells) by using one tenth of the quantities given under *Cuvette Assay*. The fluorescence reader is adjusted to the wavelengths given under *Calibration Graph*. If a time-resolving reader is available, adjust the lag time to >30 µs and integrate over 100 µs.

### Interferents

Phosphate and citrate cause an increase in fluorescence intensity of EuTc and thus interfere. Detergents should be avoided if possible. Oxygen quenches to some extent; fluorescence is stronger by ~12% if air-saturated solutions are bubbled with nitrogen gas.

**Graphs:**

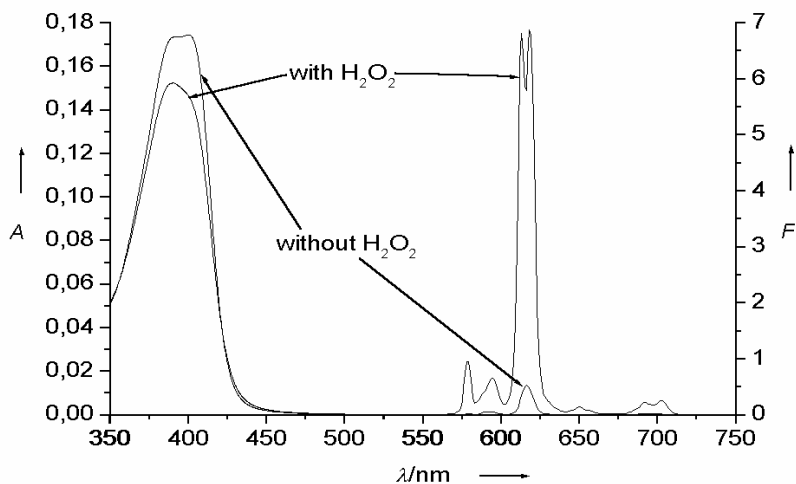


Fig. 1. Absorption and emission spectra of EuTc before and after addition of hydrogen peroxide. Fluorescence can increase by a factor of up to 19 depending in the concentration of EuTc reagent employed.

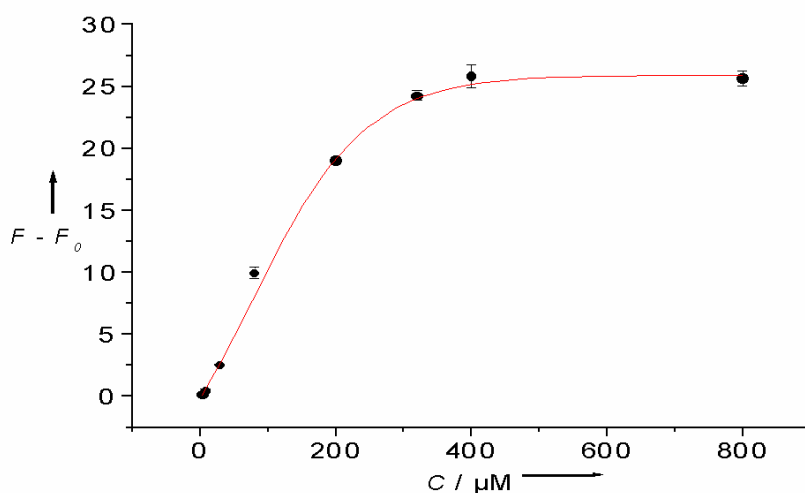


Fig. 2. Typical calibration graph, with  $(F - F_0)$  plotted against the concentration of hydrogen peroxide (HP). Here,  $F_0$  is the fluorescence intensity of the EuTc reagent before the addition of HP, and  $F$  is the fluorescence intensity after addition of HP. 1  $\mu\text{M}$  of HP is equivalent to 34 ppm of HP.