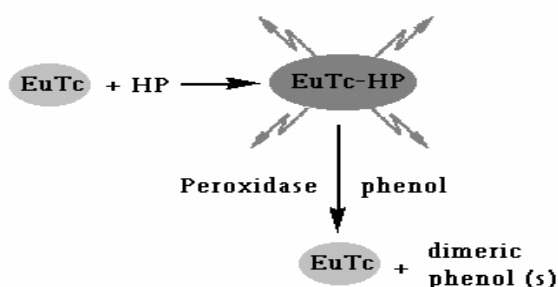


Application Note EuTc 001:

POx Assay Using the EuTc Reagent

- Scheme:** (1) $\text{EuTc} + \text{HP} \rightarrow \text{EuTc-HP}$ (strongly fluorescent)
 (2) $\text{EuTc-HP} + \text{POx} + \text{phenol} \rightarrow \text{EuTc}$ (weakly fluorescent) + products



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% H₂O₂ 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** (44 mM phenol): dissolve 207 mg of phenol (Sigma) in 50 mL of distilled water.
- Solution D** (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 E:** dissolve ~1.8 mg of POx (activity: ~145 Sigma units per mg) in 10 mL of MOPS buffer (solution D).

Recommended Protocol for Determination of the Activity of Peroxidase:

Distribute, in each well of a thermostatted (30 °C) 96-well microtiterplate, 20 – 50 µL of EuTc solution **A**, 20 µL of solution **B**, 20 µL of solution **C**, and fill up to 250 µL with MOPS buffer (solution **D**). After 10 min, add the sample containing peroxidase at an activity between 10⁻⁴ and 10⁻² Sigma units/mL, and record the decrease in fluorescence intensity for up to 20 min (depending on the enzyme activity employed). A calibration graph may be established by replacing the sample containing POx by respective aliquots of solution **E**. The solutions containing POx should also be thermostatted to 30 °C when added to the wells in order not to introduce an error due to variations in temperature. Note that phosphate causes a decrease in the fluorescence intensity of EuTc-HP and thus can interfere.

Graphs:

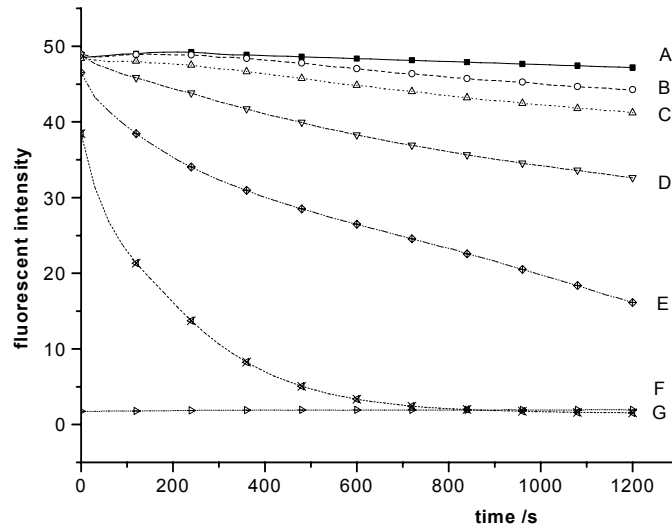


Figure 1: Kinetic determination of POx using the reagent EuTc. (A), blank; (B) to (F), increasing activities of peroxidase; (G), no POx, no H₂O₂.

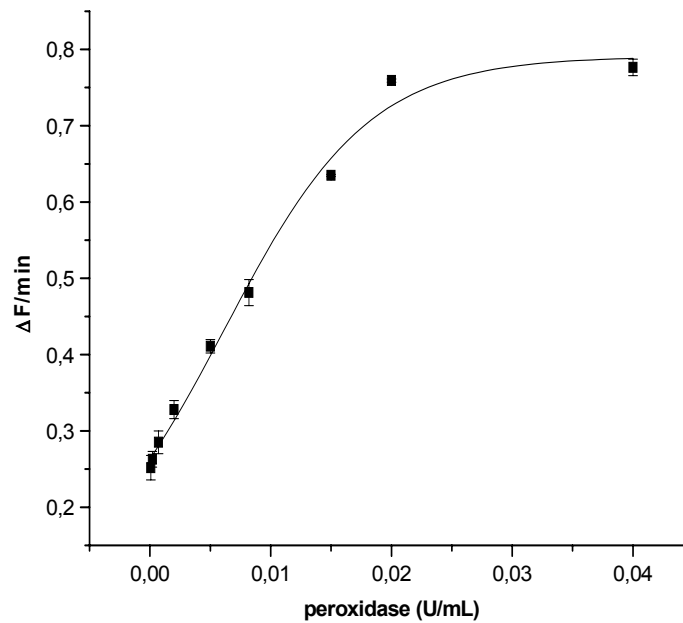


Figure 2: Typical calibration graph for peroxidase