Horseradish Peroxidase Assay

This assay uses 4-aminoantipyrine as a hydrogen donor. The reaction rate is determined by measuring an increase in absorbance at 510 nm resulting from the decomposition of hydrogen peroxide. One unit results in the decomposition of one μM of hydrogen peroxide per minute at 25°C and pH 7.0 under the specified conditions.

Reagents:

- 0.2 M potassium phosphate buffer at a pH of 7.0
- 0.0017 M hydrogen peroxide: Prepare by adding 1 ml of 30 % hydrogen peroxide to 100 ml molecular biology grade water. Further dilute 1 ml of the solution to 50 ml with 0.2 M potassium phosphate buffer.
- 0.0025 M 4-Aminoantipyrine with 0.17 M phenol: Prepare by dissolving 810 mg phenol in 40 ml molecular biology grade water. Add 25 mg of 4-aminoantipyrine and dilute to a final volume of 50 ml with molecular biology grade water.

Horseradish Peroxidase

Dissolve 1 mg of horseradish peroxidase in 1 ml molecular biology grade water. Just before use dilute further to obtain a rate of 0.02-0.04 ΔA/min.

Procedure:

1) Set spectrophotometer to 510 nm and 25°C.
2) Pipette into each cuvette as follows:

    -Phenol/aminoantipyrine solution 1.4 ml

    -0.0017 M Hydrogen peroxide 1.5 ml
3) Incubate in spectrophotometer at 25°C for 3-4 minutes to achieve temperature equilibration and establish blank rate. Add 0.1 ml of diluted enzyme and record the increase in A_{510} for 4-5 minutes. Calculate ΔA_{510}/minute from linear portion of the curve.

Calculation

$$\text{Units/mg} = \frac{\Delta A/\text{minute}}{(6.58 \times \text{mg enzyme/ml reaction mixture})}$$