SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of CATALASE
(EC 1.11.1.6)

PRINCIPLE:

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2\ H_2O + O_2$$

CONDITIONS: T = 25°C, pH = 7.0, A_{240nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer pH 7.0 at 25°C
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No, P-5379. Adjust to pH 7.0 at 25°C using 1 M KOH.)

B. 0.036% (w/w) Hydrogen Peroxide Solution (H_2O_2)(Substrate Solution)
(Prepare in Reagent A using Hydrogen Peroxide, 30% (w/w),
Sigma Prod. No. H-1009. Determine the A_{240nm} of this solution using Reagent A as a blank. The A_{240nm} should be between 0.550 and 0.520 absorbance units. Add hydrogen peroxide to increase the absorbance and Reagent A to decrease the absorbance.)

C. Catalase Solution[1]
(Immediately before use prepare a solution containing 50 - 100 units per ml in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes.

	Test	Blank
Reagent A	------	3.0
Reagent B (Substrate Solution)	2.9	------

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PROCEDURE: (continued)

Equilibrate to 25°C. Monitor the A_{240nm} until constant and record the A_{240nm} using a suitably thermostatted spectrophotometer. Then add:

	Test	Blank
Reagent C (Catalase Solution)	0.1	------

Immediately mix by inversion and record the time required for the A_{240nm} to decrease from 0.45 to 0.40 absorbance units.[2]

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(3.45)\ (df)}{(min)\ (0.1)}$$

3.45 = Corresponds to the decomposition of 3.45 micromoles of hydrogen peroxide in a 3.0 ml reaction mixture producing a decrease in the A_{240nm} from 0.45 to 0.40 absorbance units
df = Dilution factor
min = Time in minutes required for the A_{240nm} to decrease from 0.45 to 0.40 absorbance units
0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will decompose 1.0 µmole of H_2O_2 per minute at pH 7.0 at 25°C, while the H_2O_2 concentration falls from
10.3 mM to 9.2 mM. The rate of disappearance of H_2O_2 is followed by observing the rate of decrease in the absorbance at 240 nm.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.035% (w/w) hydrogen peroxide, and 5 - 10 units catalase.

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REFERENCES:

Beers, R.F. Jr. and Sizer, I.W. (1952) *Journal of Biological Chemistry* **195**, 133-140

Stern, K.G. (1937) *Journal of Biological Chemistry* **121**, 561-572

NOTES:

1. To obtain the proper activity, catalase should be completely dissolved before assaying. Catalase products supplied as a crystalline suspension may require incubation at 37°C upon dilution to obtain complete dissolution.

2. The initial A_{240nm} will exceed 0.450 absorbance units and start to decrease. Start timing the reaction when the A_{240nm} reaches 0.450 absorbance units.

3. This assay is based on the cited references.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.