Enzymatic Assay of PEROXIDASE, INSOLUBLE

PRINCIPLE:

\[
\text{H}_2\text{O}_2 + \text{Pyrogallol} \xrightarrow{\text{Peroxidase}} 2\text{H}_2\text{O} + \text{Purpurogallin}
\]

(Donor) \quad (Oxidized Donor)

CONDITIONS: \quad T = 30^\circ\text{C}, \quad \text{pH} \ 6.0, \quad A_{420\text{nm}}, \quad \text{Light path} = 1 \text{ cm}

METHOD: \quad \text{Continuous Spectrophotometric Rate Determination}

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 6.0 at 30^\circ\text{C}

(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous.
Adjust to pH 6.0 at 30^\circ\text{C} with 1 M KOH.)

B. 0.50% (w/w) Hydrogen Peroxide Solution (H\textsubscript{2}O\textsubscript{2})

(Prepare 50 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution.
PREPARE FRESH.)

C. 5.0% (w/v) Pyrogallol Solution (Pyrogallol)

(Prepare 50 ml in deionized water using Pyrogallol, PREPARE FRESH AND KEEP FROM LIGHT.)

D. Peroxidase Insoluble Enzyme Suspension (Insol Enz)

(Place sample on a small Buchner funnel. Using a suction flask and a light rate of suction, wash the agarose resin with about 10 times the sample volume of deionized water. Dry the moist gel with suction until the top of the packed gel cracks. Weigh the moist gel and place it into an appropriate container. Add one ml of deionized water per mg of sample gel. Immediately prior to assay dilute 0.1 ml of gel suspension to 2.1 ml with cold deionized water.)
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PROCEDURE:

Pipette (in milliliters) the following reagents into a suitable container while continuously mixing with a magnetic stirrer.

<table>
<thead>
<tr>
<th>Test</th>
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<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>3.30</td>
</tr>
<tr>
<td>Reagent B (H2O2)</td>
<td>1.70</td>
</tr>
<tr>
<td>Reagent C (Pyrogallol)</td>
<td>3.30</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>21.70</td>
</tr>
</tbody>
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Equilibrate to 30°C. Then add:

<table>
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<td>Reagent D (Insol Enz)</td>
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Immediately filter enough of the reaction mix (3 ml) through a 0.45 µm syringe filter into a suitable cuvette and record the \( r_{A_{420\text{nm}}} \) using a suitably thermostatted spectrophotometer\(^1\). This step should be repeated at least 4 times over seven minutes.

CALCULATIONS:

\[
\text{Units/g agarose} = \frac{(r_{A_{420\text{nm}}/\text{min}})(30.1)(21)(1000)}{(2.64)(0.1)(15.7)}
\]

30.1 = Total volume (in milliliters) of the assay
21 = Dilution factor
1000 = Conversion factor from mg to g
2.64 = Millimolar extinction coefficient\(^2\) of purpurogallin at 420nm
0.1 = Volume (in milliliter) of enzyme used
15.7 = mg dry agarose/ml of suspension

UNIT DEFINITION:

One unit will form 1.0 µmole of purpurogallin from pyrogallol per minute at pH 6.0 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 30.1 ml reaction mix, the final concentrations are 11 mM potassium phosphate, 0.03% (w/w) hydrogen peroxide, 0.55% (w/v) pyrogallol, and 0.075 mg of dry agarose peroxidase, insoluble.
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REFERENCES: