TECHNICAL INFORMATION

Catalog Number: 195271, 195272, 195273

**Lactoperoxidase**

**Molecular Weight:** 77500.22

**CAS #:** 9003-99-0

**Synonyms:** Iodide:hydrogen peroxide oxidoreductase; Iodide peroxidase

**Physical Description:** Brown chunks and lyophilized powder

**Source:** Bovine milk

**Form:** Freeze-dried powder

**Unit Definition:**

*Definition One:* One unit is equal to the formation of one micromole of triiodide per minute at 25°C and pH 7.0 in sodium phosphate buffer.

*Definition Two:* One unit will form 1.0 mg of purpurigallin from pyrogallol in 20 seconds at pH 6.0 and 20°C.

**E.C. #:** 1.11.1.8

**Purity Index:** $A_{412}/A_{280} = 0.93-0.96.1$

**Extinction Coefficient:** $E^{1\%}_{412} = 13.9$

**Inhibitors:** Lactoperoxidase in inhibited by hydrazines.2 Cyanide may also inhibit the enzyme.5

**Solubility:** Soluble in distilled water or dilute buffer

**Description:** Lactoperoxidase catalyzes the hydrogen peroxide oxidation of iodide:

$$2I^- + H_2O_2 + 2H^+ \rightarrow I_2 + 2H_2O$$

Lactoperoxidase, in the presence of peroxide, catalyzes the oxidation of many phenols and aromatic amines (pyrogallol, ascorbate, guiacol, etc.). Morrison and Bayse18 indicate that the iodide reacts directly with the heme group, the complex then iodinates the substrate on adding $H_2O_2$.

Lactoperoxidase isolated from bovine milk is identical to that formed in bovine lacrimal and salivary glands.15,16 It has been reported that lactoperoxidase may be important in controlling bacterial flora.3,8,17 Lactoperoxidase is typically used for labelling proteins with radioiodine.4,6,7,9,10,12,14,18 For membrane studies the large lactoperoxidase molecule limits labelling to the exposed surface.

**Composition:** Lactoperoxidase is a glycoprotein with a single hemin prosthetic group per molecule.11 It may consist of two isozymes.19,20,21,22

**Assay Method 1:**

The assay procedure is a slight modification of that described by Morrison.13 The reaction velocity is determined by measuring the increase in $A_{350}$ resulting from the production of triiodide.

**Reagents Needed:**
A. 0.033 M Sodium phosphate buffer, pH 7.0
B. 0.005 M Potassium iodide in phosphate buffer (Reagent A).
C. 0.090 M Hydrogen peroxide. Prepare by diluting 0.1 ml 30% hydrogen peroxide to 10 ml with reagent grade water.
D. Enzyme: Dissolve at one mg/ml in phosphate buffer (Reagent A). Immediately prior to use, dilute further in phosphate buffer to obtain a rate of 0.02-0.04 DA/minute.

Procedure:
Set spectrophotometer at 25°C and 350 nm. Prepare reaction mixture by diluting 0.15 ml of Reagent C (hydrogen peroxide solution) to 30 ml with Reagent B (potassium iodide solution). Store no longer than 30 minutes at room temperature. Pipette 3.0 ml reaction mixture into cuvette and incubate at 25°C for 3-4 minutes to achieve temperature equilibrium and establish a blank rate, if any. Add 0.01 ml diluted enzyme and record increase in A$_{350}$ for 3-4 minutes. Calculate DA$_{350}$/minute from initial linear portion of curve. Reaction remains linear for no longer than 1-2 minutes.

Calculation:

Units/mg = \frac{\Delta A_{350} / \text{min}}{26 \times \text{mg enzyme/ml reaction mixture}}

Assay Method 2:$^{23}$

Method: A continuous spectrophotometric rate determination.

\[
\text{H}_2\text{O}_2 + \text{Pyrogallol (donor)} \xrightarrow{\text{Lactoperoxidase}} 2\text{H}_2\text{O} + \text{Pyrogallin (oxidized donor)}
\]

Reagents Needed:
A. 100 mM Potassium phosphate buffer, pH 6.0 at 20°C. Prepare 100 ml in deionized water using potassium phosphate monobasic anhydrous. Adjust to pH 6.0 at 20°C with 1 M KOH.
B. 0.5% (w/w) hydrogen peroxide solution. Prepare 50 ml in deionized water using 30% hydrogen peroxide solution. Prepare Fresh for each use.
C. 5% (w/v) pyrogallol solution. Prepare 10 ml in deionized water using pyrogallol. Prepare fresh for each use and keep from light.
D. 0.1% (w/v) bovine serum albumin solution (enzyme diluent). Prepare 50 ml in Reagent A (potassium phosphate buffer).
E. Lactoperoxidase Enzyme Solution. Immediately before use, prepare a solution containing 0.4-0.7 unit/ml of enzyme in cold Reagent D (BSA solution).

Procedure:
Pipette the following reagents into suitable cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>2.10 ml</td>
<td>2.10 ml</td>
</tr>
<tr>
<td>Reagent A</td>
<td>0.32 ml</td>
<td>0.32 ml</td>
</tr>
<tr>
<td>Reagent B</td>
<td>0.16 ml</td>
<td>0.16 ml</td>
</tr>
<tr>
<td>Reagent C</td>
<td>0.32 ml</td>
<td>0.32 ml</td>
</tr>
<tr>
<td>Reagent D</td>
<td>0.0 ml</td>
<td>0.10 ml</td>
</tr>
<tr>
<td>Reagent E</td>
<td>0.10 ml</td>
<td>0.0 ml</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 20°C. Monitor the A$_{420}$ until constant, using a suitably thermostated spectrophotometer. Then add:
Immediately mix by inversion and record the increase in $A_{420}$ for approximately 5 minutes. Obtain the $DA_{420}/20$ seconds using the maximum linear rate (0.16-0.28; the enzyme concentration may have to be modified in order to fit this rate) for both the Test and Blank.

**Calculation:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{420}/20 \text{ sec Test} - \Delta A_{420}/20 \text{ sec Blank}) (3) (df)}{(12) (0.1)}$$

where:

- $\Delta A_{420}$ = absorbance change at 420 nm
- sec = seconds
- V = volume (in milliliters) of assay
- df = dilution factor
- 0.1 = volume (in milliliters) of enzyme used

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

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

**Availability:**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>195271</td>
<td>Lactoperoxidase; activity approximately 80-100 units/mg protein; lyophilized</td>
<td>1 mg, 5 mg, 10 mg</td>
</tr>
<tr>
<td>195272</td>
<td>Lactoperoxidase; activity approximately 60-80 units/mg protein; lyophilized</td>
<td>1 mg, 5 mg, 10 mg, 25 mg, 100 mg</td>
</tr>
<tr>
<td>195273</td>
<td>Lactoperoxidase; activity not less than 200 units/mg protein; protein approximately 90-95%</td>
<td>5 mg, 10 mg, 25 mg, 100 mg</td>
</tr>
</tbody>
</table>

**References:**

- Morrison, M., "Iodination of tyrosine: Isolation of lactoperoxidase (bovine)
  " in *Methods in Enzymology*, XVIIA, (Tabor, H. and