Active Native Bovine Lactoperoxidase

Cat. No. Lactoperoxidase-18B **Lot. No.** (See product label)

SPECIFICATION

Product Overview	LPO is a glycoprotein with a single hemin prosthetic group per molecule. It may consist of two isozymes.
Species	Bovine
Source	Milk
Description	Lactoperoxidase (LPO) is a glycoprotein with a hemin prosthetic group which may occur as a mixture of two isozymes. It has a molecular weight of 77,500 daltons. LPO catalyzes the hydrogen peroxide oxidation of iodide according to the following reaction: $2I^- + H_2O_2 + 2H^+ \rightarrow I_2 + 2H_2O$. Iodide reacts directly with the heme group; upon addition of H_2O_2 the complex iodinates the substrate. LPO is inhibited by hydrazines. The assay procedure has been updated from that of Morrison to an ABTS®/H_2O_2 based method with increased sensitivity and reproducibility.
Form	A lyophilized powder.
Bio-activity	≥35 units/mg dry weight ABTS
Molecular Mass	77.5 kDa
Purity	Chromatographically purified. A412/A280 = 0.93-0.96
Storage	Store at -20 centigrade.

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EC	1.11.1.7
CAS	9003-99-0
Unit definition	One Unit reduces one micromole of hydrogen peroxide per minute at 25 centigrade, pH 6.0. Note: This unit definition is based upon a new assay method. The previous method yielded approximately 2.8 times higher results.
Usage	Method: The assay procedure is a modification of that described by Putter and Becke r. The increase in the absorbance at 405 nm resulting from the oxidation of 2,2-azino-di(3-ethyl-bnzothiazoline-6-sulphonic acid), diammonium salt, ABTS. Two moles of A BTS are oxidized for every mole of peroxide that is reduced. Reagents: 0.08 M Sodiu m-Potassium Phosphate Buffer pH 6.0: Prepare by adjusting the pH of 0.08 M sodium phosphate, dibasic, to 6.0 using 0.08 M potassium phosphate, monobasic. Sodium P otassium Phosphate Buffer, containing 1 mg/mL Bovine Serum Albumin: Dissolve BS A at a concentration of mg/ml in an aliquot of the above buffer. This will be used as en zyme diluent. 0.0017 M Hydrogen peroxide. Prepare by diluting 1 ml of 30% hydrogen peroxide to 100 ml with reagent grade water. Further dilute 1 ml of this solution to 50 ml with reagent grade water. Prepare fresh daily. 0.051 M ABTS: Dissolve ABTS in 0. 08 M sodium phosphate buffer, pH 6.0 Enzyme: Dissolve at one mg/ml in sodium-pho sphate buffer. Immediately prior to use, dilute further in phosphate buffer with BSA to obtain a rate of 0.008-0.025 DA/minute. Keep all dilutions on ice. Procedure: Set spec trophotometer at 25 centigrade and 405 nm. Pipette into each cuvette 2.65 ml sodiu m- phosphate buffer, 0.15 mL hydrogen peroxide, and 0.10 ml ABTS. Incubate in spe ctrophotometer at 25 centigrade for 3-5 minutes to achieve temperature equilibration and establish a blank rate, if any. Add 0.10 ml diluted enzyme and record the increas e in A405 for 4-6 minutes. Calculate the rate from the maximum initial linear portion of the curve. The reaction is linear for approximately two minutes. Calculation: (Units/m g)=(ΔA350/min×3.0×dilution)/(18.6×2×sample volume) 18.6= extinction coefficient of ABTS at 405nm 2 ABTS: 1 H2O2

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Extinction Coefficient	13.9
Inhibitors	LPO is inhibited by hydrazines. Dolman et al. (1968) report on the kinetics of cyanide inhibition.

GENE INFORMATION

Official Symbol	Lactoperoxidase
Synonyms	Lactoperoxidase

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