MP Biomedicals, LLC
29525 Fountain Parkway
Solon, Ohio 44139
Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
mailto: biotech@mpbio.com
web: http://www.mpbio.com

TECHNICAL INFORMATION

Catalog Number: 100978
Phosphodiesterase I
CAS #: 9025-82-5
Source: *Crotalus adamanteus* venom
Form: Lyophilized in vials containing approximately 5 mg dry weight and approximately 100 units/vial (20-40 units/mg dry weight).
Preparation: Preparation of Williams, Sung and Laskowski, *J. Biol. Chem.*, **v. 236**, 1130 (1961). Further treated to inactivate contaminating 5'-nucleotidase according to Sulkowski and Laskowski, *Biochim. Biophys. Acta*, **v. 240**, 443 (1971).
Description: Phosphodiesterase I successively hydrolyzes 5'-mononucleotides from 3'-hydroxy-terminated ribo- and deoxyribo-oligonucleotides.[1,2]
ADP-ribosylated proteins are cleaved at the pyrophosphate linkages by venom phosphodiesterases to yield phosphoribosyl-AMP.[3]
The enzyme has been widely utilized as a tool for structural and sequence studies of nucleic acids.[4]
Molecular Weight: 115,000.[5]
Composition: A glycoprotein which binds concanavalin A.[6]
Optimum pH: 9.8 to 10.4.5
Activators: The enzyme has an absolute requirement for Mg^{2+}; Philipps[5] indicates an optimum concentration of 15 mM.
Inhibitors: Reducing agents such as gluctathione, cysteine and ascorbic acid.[1] It is completely inhibited by 5 mM EDTA while ATP, ADP and AMP are partial inhibitors.
Assay
Method: The assay is based on that of Razell and Khorana[7] where the reaction velocity is determined by an increase in absorbancy at 400 nm resulting from the hydrolysis of p-nitrophenyl thymidine-5'-phosphate.
Unit Definition: One unit hydrolyzes 1 umole of p-nitrophenyl thymidine-5-phosphate per minute at pH 8.9 and 25°C.
Reagents:

– 0.11 M Tris-HCl buffer, pH 8.9, with 0.11 M NaCl and 15 mM $MgCl_2$. (Tris/Salts buffer).
– 5 mM p-Nitrophenyl thymidine-5'-phosphate. The purity of commercial preparations varies somewhat and should be considered in preparing this reagent.
– Enzyme: Dissolve at one mg/ml in Tris/Salts buffer. Immediately prior to use, dilute further in Tris/Salts buffer to a concentration of 0.1 to 0.4 units/ml

Procedure:
Set spectrophotometer at 400 nm and 25°C. Pipette into microcuvettes 0.9 ml Tris/Salts buffer and 0.1 ml 5 mM p-Nitrophenyl thymidine-5'-phosphate.
Incubate cuvettes in spectrophotometer for 3-5 minutes to reach temperature equilibrium and establish blank rate, if any. Add 10 microliters of diluted enzyme and record increase in A_{400} for 3-5 minutes. The reaction remains linear until A_{400} reaches about 1.2. Calculate DA400/min from initial linear portion of absorbance curve.
Calculation:

$$\text{Units/mg} = \frac{\Delta A_{400}/\text{min}}{16^{*} \times \text{mg enzyme/ml reaction mixture}}$$

* 16 is the extinction coefficient of p-nitrophenol determined in this laboratory under these conditions.
References:

– Razell, W.E., in Methods in *Enzymology, v. VI*, (Colowick, S.P. and Kaplan, N.O., eds.) p. 236, Academic Press, NY (1963).
– Laskowski, M., Sr., in *The Enzymes, v. IV*, **3rd Ed.**, (Boyer, P.D., ed.), p. 313, Academic Press, NY (1971).
– Hayaishi, O., *Trends Biochem. Sci.*, **v. 1**, 9 (1976).
– Ho, N.W.Y. and Gilham, P.T., *Biochim. Biophys. Acta*, **v. 308**, 53 (1973).
– Philipps, G.R., *Hoppe-Seyler's Z. Physiol. Chem.*, **v. 356**, 1085 (1975).
– Sulkowski, E., and Laskowski, M., Sr., *Biochem. Biophys. Res. Comm.*, **v. 57**, 463 (1974).
– Razell, W.E. and Khorana, H.G., *J. Biol. Chem.*, **v. 234**, 2105 (1959).