Pyrophosphatase, Inorganic (*E. coli*)

1-800-632-7799
info@neb.com
www.neb.com

M0361S 003160618061

M0361S

10 units	**100 U/ml**	**Lot: 0031606**
RECOMBINANT	**Store at –20°C**	**Exp: 6/18**

Description: Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate.

$$P_2O_7^{-4} + H_2O \rightarrow 2HPO_4^{-2}$$

Source: PPase is prepared from an *E. coli* strain containing a clone of the *E. coli* inorganic pyrophosphatase gene.

Applications:

- Increasing RNA yield in transcription reaction; enhancing DNA replication.

Supplied in: 20 mM Tris-HCl, 100 mM NaCl, 1 mM Dithiothreitol, 0.1 mM EDTA, 50% Glycerol, pH 8.0 @ 25°C. Store at –20°C.

Unit Definition: One unit is the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions (a 10 minute reaction at 25°C in 20 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$ and 2 mM PPi).

Quality Assurance: This enzyme is validated in an *in vitro* RNA synthesis reaction.

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 0.1 unit of the enzyme with 40 ng of 300 base RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by denaturing PAGE analysis.

Exonuclease Assay: Incubation of a 50 µl reaction containing 1 unit of the enzyme with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C released < 0.5% of the total radioactivity.

Endonuclease Activity: Incubation of a 10 µl reaction containing 0.1 unit of the enzyme with 300 ng of supercoiled plasmid for 16 hours at 37°C produced less than 10% nicked or linear molecules as determined by agarose gel electrophoresis.

Alkaline Phosphatase Activity: This colorimetric assay tests for the presence of alkaline phosphatase which removes 5´ phosphates from DNA, RNA, rNTPs and dNTPs. One unit of alkaline phosphatase is defined as the amount of enzyme that hydrolyzes 1 µmole of *p*-nitrophenylphosphate in 1 minute. When 2 units of the enzyme are incubated under standard alkaline phosphatase assay conditions (1 ml reaction containing 1 M diethanolamine-HCl (pH 9.8), 0.5 mM $MgCl_2$ and 10 mM *p*-nitrophenylphosphate at 37°C for 1 hour), < 0.0001 unit of alkaline phosphatase activity is detected.

Usage Note: Use 1–3 units per ml high yield *in vitro* RNA synthesis reaction.

Heat Inactivation: No

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices

CERTIFICATE OF ANALYSIS

Pyrophosphatase, Inorganic (*E. coli*)

1-800-632-7799
info@neb.com
www.neb.com

M0361S 003160618061

M0361S

10 units | 100 U/ml | Lot: 0031606
RECOMBINANT | Store at –20°C | Exp: 6/18

Description: Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate.

$$P_2O_7^{-4} + H_2O \rightarrow 2HPO_4^{-2}$$

Source: PPase is prepared from an *E. coli* strain containing a clone of the *E. coli* inorganic pyrophosphatase gene.

Applications:

- Increasing RNA yield in transcription reaction; enhancing DNA replication.

Supplied in: 20 mM Tris-HCl, 100 mM NaCl, 1 mM Dithiothreitol, 0.1 mM EDTA, 50% Glycerol, pH 8.0 @ 25°C. Store at –20°C.

Unit Definition: One unit is the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions (a 10 minute reaction at 25°C in 20 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$ and 2 mM PPi).

Quality Assurance: This enzyme is validated in an *in vitro* RNA synthesis reaction.

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 0.1 unit of the enzyme with 40 ng of 300 base RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by denaturing PAGE analysis.

Exonuclease Assay: Incubation of a 50 µl reaction containing 1 unit of the enzyme with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C released < 0.5% of the total radioactivity.

Endonuclease Activity: Incubation of a 10 µl reaction containing 0.1 unit of the enzyme with 300 ng of supercoiled plasmid for 16 hours at 37°C produced less than 10% nicked or linear molecules as determined by agarose gel electrophoresis.

Alkaline Phosphatase Activity: This colorimetric assay tests for the presence of alkaline phosphatase which removes 5´ phosphates from DNA, RNA, rNTPs and dNTPs. One unit of alkaline phosphatase is defined as the amount of enzyme that hydrolyzes 1 µmole of *p*-nitrophenylphosphate in 1 minute. When 2 units of the enzyme are incubated under standard alkaline phosphatase assay conditions (1 ml reaction containing 1 M diethanolamine-HCl (pH 9.8), 0.5 mM $MgCl_2$ and 10 mM *p*-nitrophenylphosphate at 37°C for 1 hour), < 0.0001 unit of alkaline phosphatase activity is detected.

Usage Note: Use 1–3 units per ml high yield *in vitro* RNA synthesis reaction.

Heat Inactivation: No

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices

Covered under U.S. Patent No. 5,861,296.

NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

CERTIFICATE OF ANALYSIS