## Protein Phosphatase Inhibitor 2 (I-2)



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

# P0755S



50 μg 1 mg/ml Lot: 0061204 RECOMBINANT Store at -20°C Exp: 4/14

**Description:** Protein Phosphatase Inhibitor-2 (I-2) specifically and instantaneously inhibits the catalytic subunit of type I protein phosphatase (PP1) at nanomolar concentrations. In contrast, the ihibition of native forms of PP1, type 1 holoenzymes, is time dependent and requires much more I-2. I-2 inhibits PP1 from mammals, yeast, and flies in an identical manner and can be used for identification of type I protein phosphatases from many different species (1-3).

I-2 is a 204 amino acid heat stable protein. Recombinant I-2 has identical inhibitory properties as native rabbit muscle I-2 (2).

I-2 is functionally tested in the inhibition assays of PP1 (NEB #P0754).

**Source:** Isolated from a strain of *E. coli* that carries the coding sequence for rabbit skeletal muscle I-2 polypeptide under the control of T7 expression system (kindly provided by Dr. A.A. DePaoli-Roach) (3).

Supplied in: 50 mM HEPES (pH 7.0 @ 25°C), 0.1 mM EGTA, 0.01% Brij 35 and 50% glycerol.

**Molecular Weight:** 22,800 daltons. The apparent molecular weight of I-2 is 31 kDa on SDS-PAGE (1,2).

**Purity:** I-2 has been purified to > 95% homogeneity as determined by SDS-PAGE and Coomassie Blue staining.

## **Quality Control Assays**

Protease Activity: After incubation of 5  $\mu$ g of I-2 with a standardized mixture of proteins for 2 hours at 30°C, no proteolytic activity could be detected by SDS-PAGE.

Phosphatase Activity: After incubation of 5  $\mu$ g of I-2 with 50 mM  $\rho$ -Nitrophenyl Phosphate (NEB #P0757) for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

**Notes On Use:** Avoid repeated freeze/thaw cycles. Recommended long term storage –70°C.

PP1 or cell extracts should be diluted so that the concentration of phosphatase activity is less than 0.1 U/ml in the assays. One unit is the amount of PP1 that hydrolyzes 1 nmol of substrate in 1 minute. The diluted extracts must be preincubated with I-2 for 10–15 minutes prior to assay in order to achieve complete inhibition (1).

#### References:

- 1. Cohen, P. (1991) *Methods in Enzymology*, 201, 389–398.
- 2. Park, I., et al. (1994) *J. Biol. Chem.* 269, 944–954.
- 3. Connor, J.H., et al. (2000) *J. Biol. Chem.* 275, 18670–18675.

### **Companion Products:**

Protein Phosphatase I

#P0754S 100 units #P0754L 500 units

CERTIFICATE OF ANALYSIS

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# P0755S 0061204140

RX

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RECOMBINANT Store at -20°C Exp: 4/14

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CERTIFICATE OF ANALYSIS