

**Protein
Phosphatase
Inhibitor 2 (I-2)**



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



P0755S 007131015101

P0755S



50 µg 1 mg/ml Lot: 0071310

RECOMBINANT Store at -20°C Exp: 10/15

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 inhibition 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 µg 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 µg of I-2 with 50 mM *p*-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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