Dephosphorylating DNA with CIP:

1. Suspend DNA in 1X NEBuffer (0.5 µg/10 µl).
2. Add 0.5 unit/µg vector DNA.
3. Incubate 60 minutes at 37°C.
4. Purify DNA by gel purification, spin-column purification or phenol extraction.

Dephosphorylating Proteins with CIP:

CIP can be used to release phosphate groups from phosphorylated tyrosine, serine and threonine residues in proteins, using the following protocol:

1. Add protien/lysate to 1x NEB buffer (0.5-1.0 ug/10ul)
2. Add 0.5 - 1.0 unit/ug protein.
3. Incubate 60 minutes at 37°C.

Usage notes:

10 units of CIP removes 100% phosphates in phosphorylated myelin basic protein (phospho-MyBP) in 60 minutes at 37°C in a 50 µl reaction. The concentration of phospho-MyBP is 10 µM with respect to phosphate.

Protein Serine/threonine Phosphates (PSP) activity is assessed on phospho-MyBP phosphorylated exclusively on serine/threonine residues with cAMP-dependent Protein Kinase using the Protein Serine/Threonine Phosphatase Assay System (NEB#P0780). The Protein Tyrosine Phosphates (PTP) activity is assessed on phospho-MyBP phosphorylated exclusively on tyrosine residues with Abl Protein Tyrosine Kinase using Protein Tyrosine Phosphatase Assay System (NEB #P0785).

If the source of phosphorylated protein is a crude extract of cells or tissue it is very important to use the appropriate proteinase inhibitors in the lysis buffer and to use short incubation times for dephosphorylation.

Note that optimal incubation time and enzyme concentration must be determined empirically for a particular substrate. CIP activity is optimal in NEBuffers 2, 3 or 4 and should not be use in NEBuffer 1 (50% activity). Slightly lower activity may be observed in any reaction buffer containing a total salt concentration of less than 50 mM, under these conditions a 2-fold excess of CIP is recommended.

Inhibition: 10 mM sodium orthovanadate inhibits CIP activity (10 units) by 90% and 50 mM EDTA inactivates CIP (10 units) by 100%.