In -20°C freezer

Shrimp Alkaline Phosphatase

Shrimp Alkaline Phosphatase is isolated from cold-living North Sea shrimp. It catalyzes the hydrolysis of many phosphate esters, including 5'-nucleotides, DNA and RNA. Its properties are similar to those of Calf Intestine Alkaline Phosphatase with the exception that it is heat-labile and has somewhat different buffer requirements. It is purified to homogeneity, and appears to be a single polypeptide on SDS polyacrylamide gels with a molecular weight of about 59,000. It is free of nucleases, making it useful for molecular biology.

Unit Definition

One unit is the amount of enzyme required to catalyze the hydrolysis of one micromole of p-nitrophenyl-phosphate per minute at pH9.6 and 25°C in a glycine/NaOH buffer. (This is the same as the unit definition for Calf-Intestine Alkaline Phosphatase).

Use of Shrimp Alkaline Phosphatase

The rate of removal of the terminal 5' phosphate from double-stranded DNA depends on the structure of the terminus. Termini with 5' protruding ends are more reactive than those with blunt ends or those with 5'-recessed ends. The reaction rate also depends on the temperature and magnesium concentration. USB recommends that dephosphorylation of DNA be done at 37°C in the following buffer:

20mM Tris.HCl pH 8.0
10mM $MgCl_2$
20µg/ml DNA

The amount of enzyme depends on the the kind of termini and the amount of DNA. For a typical reaction using 1.0 pmol of DNA termini (2.5µg of 3Kb plasmid), the following amounts were found to be effective:

Terminus	Units of phosphatase (1 hour, 37°C)
5'-Protruding	0.1 units
Blunt	0.2 units
5'-Recessed	0.5 units

NOTE: These are minimum effective amounts. It may be prudent to use more enzyme or longer incubation times to assure complete dephosphorylation.