invitrogen
by life technologies

Heat Inactivated Alkaline Phosphatase

Cat. no.: A14322 **Size:** 400 reactions
Conc.: 5 U/µL **Store at –20°C** (non-frost-free)

Pub. Part no. 100014009 MAN0005252 **Rev. Date:** 17 Oct 2011

Description

Heat Inactivated Alkaline Phosphatase is a phosphomonoesterase that hydrolyzes 3′ and 5′ phosphates from DNA and RNA. It is suitable for removing 5′ phosphates prior to end-labeling and for dephosphorylating vectors prior to insert ligation. It is sensitive to inhibition by micromolar amounts of inorganic phosphate. Zinc is contained in the enzyme storage buffer and is necessary for optimal enzyme activity.

Component	Amount
Heat Inactivated Alkaline Phosphatase	2000 units
10X Reaction Buffer	1 mL

Unit Definition

Five units dephosphorylates 1 µg of pUC19 DNA cut with HindIII (5′ protruding), HincII (blunt) or PstI (3′ protruding) in 30 minutes at 37°C.

Buffer Composition

Storage buffer: 10 mM Tris-HCl (pH 7.5); 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 1 mM DTT, 50% (v/v) glycerol.

10X Reaction Buffer: 100 mM Tris-HCl (pH 7.5); 100 mM $MgCl_2$, 10 mM DTT.

Intended Use: For research use only. Not for human or animal therapeutic or diagnostic use.

Dephosphorylation Protocol

This protocol allows for the dephosphorylation of DNA directly in restriction endonuclease buffer in the presence of the restriction endonuclease. 1 µL of Heat Inactivated Alkaline Phosphatase can be used to dephosphorylate 1–5 µg of DNA.

1. Add 1 µL (5U) of enzyme to the restriction digestion reaction. Use at least 1 µL of enzyme for every 50 µL (or fraction thereof) of the reaction.
2. Incubate for 15 minutes at 37°C for cuts resulting in 5′ overhangs or blunt ends. For cuts resulting in 3′ protruding ends, incubate for 60 minutes at 37°C.
3. Heat inactivate for 5 minutes at 65°C (or as necessary to inactivate the restriction enzyme originally used to cut the DNA).
4. Ligate insert and vector using T4 DNA Ligase (Cat. no. 15224017), or ExpressLink™ T4 DNA Ligase (Cat. no. A13726) and proceed with cloning procedure.

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

©2011 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™