

Stop Cloning or Subcloning Artifacts!

Remove All Chromosomal DNA From Your Plasmid, Cosmid or BAC Cloning Vector Preps Using Plasmid-Safe™ DNase

Preparations of plasmid, cosmid, fosmid and BAC cloning vectors are frequently contaminated with fragments of bacterial genomic DNA that are generated during alkaline lysis. Commercial purification columns and even CsCl centrifugation do not effectively remove all these contaminants. Ultimately, the contaminating DNA fragments are ligated into the cloning vector and result in false positives and high backgrounds.

Plasmid-Safe™ ATP-Dependent DNase provides a powerful, fast and easy method to completely remove all traces of bacterial chromosomal DNA contamination from cloning vector preparations in one hour. Plasmid-Safe is an ATP-dependent DNase that selectively digests

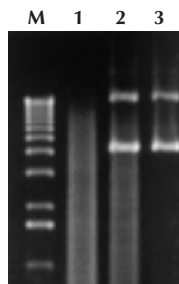


Figure 1. Use of Plasmid-Safe™ ATP-Dependent DNase to remove contaminating linear DNA from plasmids. Lane 1, 3 µg of *Sma* I-digested bacterial chromosomal DNA; Lane 2, mixture of 3 µg of digested bacterial chromosomal DNA and 500 ng of uncut plasmid before Plasmid-Safe DNase treatment; Lane 3, mixture of chromosomal DNA and plasmid DNA after Plasmid-Safe treatment for 30 minutes at 37°C; M, DNA ladder.

linear double-stranded DNA but has no activity on nicked, closed-circular dsDNA or supercoiled DNA. Therefore, Plasmid-Safe DNase is recommended as a final purification step for all cloning vector preparations.

Plasmid-Safe™ ATP-Dependent DNase	
E3101K-F83	1,000 U
E3105K-F83	5,000 U
E3110K-F83	10,000 U

Simplify Dephosphorylation of Your Cloning Vectors Using Heat-Labile HK™ Phosphatase

Derived from an Antarctic bacterium, HK™ (Heat-Killable) Phosphatase greatly simplifies dephosphorylation of cloning vectors prior to ligation of the DNA insert. The enzyme is as effective as calf intestinal alkaline phosphatase (CIP) and

bacterial alkaline phosphatase (BAP) in removing phosphates from protruding 5'-end of dsDNA generated by many restriction endonucleases (e.g., *Bam*H I, *Eco*R I, *Hind* III, etc.). However, unlike CIP and BAP, HK Phosphatase is completely and irreversibly inactivated by incubation at 70°C for 15 minutes (Table 1). Note: we do not recommend using HK Phosphatase for blunt ends and 3'-protruding ends.

Unit Definition: One Molecular Biology Unit (MBU) dephosphorylates 1 µg of *Hind* III-digested pUC19 in one hour at 30°C in 1X TA Buffer.

Reference

- Hoffman, L.M. and Jendrisak, J. (1990) *Gene* **88**: 97.

Table 1. HK™ Phosphatase is completely inactivated after heat treatment at 70°C for 15 minutes.

Phosphatase	% Activity After Heating
HK Phosphatase	0
BAP	41
CIP	62

Each phosphatase was incubated at 70°C for 15 minutes. Remaining phosphatase activity was then determined by incubation with 5'-³²P-labeled RNA.

HK Phosphatase is:

- Completely and irreversibly heat-inactivated.
- Active in most restriction enzyme buffers so that restriction enzyme digestion, dephosphorylation and DNA ligation can be performed in a single tube, without time-consuming and yield-reducing phenol extraction or ethanol precipitation.
- Active in removing the 5'-phosphate from DNA and RNA.

HK™ Phosphatase

H92025-F83	25 MBU*
H92050-F83	50 MBU*
H92100-F83	100 MBU*
Supplied at 1 U/µl. Includes 10X TA Buffer and 0.1 M CaCl ₂ .	
*MBU = Molecular Biology Unit	