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Plasmid-Safe™ ATP-Dependent DNase

Cat. Nos. E3101K, E3105K, and E3110K

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1. Introduction

Plasmid-Safe™ ATP-Dependent DNase selectively hydrolyzes linear double-stranded (ds) DNA to deoxynucleotides at slightly alkaline pH and, with a lower efficiency, linear and closed-circular single-stranded DNAs. The reaction is ATP-dependent, and does not affect closed-circular supercoiled or nicked circular dsDNAs. The enzyme can be conveniently and completely heat-inactivated by a 30 minute incubation at 70°C. Plasmid-Safe DNase is useful as a final "cleanup" of DNA preparations from plasmid and cosmid clones, to avoid the problems caused by contaminating genomic DNA.

Plasmid-Safe ATP-Dependent DNase is available in 1,000-, 5,000-, and 10,000-unit sizes at a concentration of 10 U/µl. The enzyme is supplied with a 10X Reaction Buffer and a 25-mM ATP Solution.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: Plasmid-Safe DNase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.1% Triton® X-100.

Unit Definition: One unit degrades 1 nmol of deoxynucleotides in linear dsDNA in 30 minutes at 37°C in 1X Plasmid-Safe Reaction Buffer and 1 mM ATP.

Plasmid-Safe 10X Reaction Buffer: 330 mM Tris-acetate (pH 7.5), 660 mM potassium acetate, 100 mM magnesium acetate, and 5.0 mM DTT.

ATP is required for Plasmid-Safe DNase activity and should be added to a final concentration of 1 mM.

Contaminating Activity Assays: Plasmid-Safe DNase is free of detectable RNase and double-strand-specific endonuclease activities.

3. Example Protocol

1. Isolate DNA from overnight bacterial cultures using standard mini- (1- to 2-ml), midi- (10- to 100-ml) or maxi-preparation (500- to 1,000-ml) protocols.
2. Resuspend the DNA in the appropriate amount of sterile water and set up the Plasmid-Safe DNase reaction as indicated.

For mini-preparations:

42 µl	sterile water
2 µl	25 mM ATP
5 µl	10X Reaction Buffer
1 µl	Plasmid-Safe DNase (10 U)
50 µl	total volume

For midi-preparations:

210 µl	sterile water
10 µl	25 mM ATP
25 µl	10X Reaction Buffer
5 µl	Plasmid-Safe DNase (50 U)
250 µl	total volume

For maxi-preparations:

410-420 µl	sterile water
20 µl	25 mM ATP
50 µl	10X Reaction Buffer
10-20 µl	Plasmid-Safe DNase (100-200 U)
500 µl	total volume

3. Incubate at 37°C for (see Notes below):
   30 minutes for a mini-preparation
   1-16 hours for a midi-preparation
   2-16 hours for a maxi-preparation
4. Inactivate Plasmid-Safe DNase by incubation at 70°C for 30 minutes.

Notes:

1. Treated DNA can be further purified by ethanol precipitation, spin columns, or organic extraction.
2. Precise amounts of Plasmid-Safe DNase can be added to clean up nucleic acid solutions by estimating the amount of chromosomal DNA contamination and using the following conversion: 3 U of Plasmid-Safe DNase will digest 1 µg of DNA in 30 minutes at 37°C.
3. Contaminating chromosomal DNA isolated with plasmid DNA in a typical alkaline lysis preparation is generally sufficiently nicked and sheared, making a good substrate for Plasmid-Safe DNase. Conversely, relatively intact chromosomal DNA (as expected in a gentle BAC or cosmid DNA preparation) will be degraded slowly because of only a few loci from which the exonuclease can act. To remedy this situation, you can treat the chromosomal DNA overnight with Plasmid-Safe DNase, or treat the chromosomal DNA with a restriction enzyme that does not digest the plasmid or cosmid of interest prior to Plasmid-Safe DNase digestion. Alternatively, the chromosomal DNA can be mechanically sheared either by vortex mixing or repeated pipetting through a small micropipettor tip.

4. Related Products

The following products are also available:

Cat. #	Concentration	Quantity
Ready-Lyse™ Lysozyme Solution		
R1802M		2 X 10^6 U
R1804M		4 X 10^6 U
R1810M		10 X 10^6 U
GELase™ Agarose Gel-Digesting Preparation		
G09050	1 U/µl	50 U
G09100	1 U/µl	100 U
G09200	1 U/µl	200 U
G31050	0.2 U/µl	50 U
G31200	0.2 U/µl	200 U
Includes GELase™ 50X Reaction Buffer.		
GELase™ 50X Reaction Buffer		
G191ML		1 ml
G195ML		5 ml
1 ml of 50X Buffer is sufficient for replacing the buffer in at least 5 g of gel.		
Ammonium Acetate Solution		
G1005ML	5 M	5 ml
G1025ML	5 M	5 x 5 ml
5 ml is sufficient for ethanol precipitation of DNA or RNA from 5 ml of digested gel solution.		

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