Manual

Plasmid-Safe ATP-Dependent DNase

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Plasmid-Safe™ is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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GENOMIC ANALYSIS BY LGC

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1. Introduction

Plasmid-Safe ATP-Dependent DNase selectively hydrolyses 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.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
Plasmid-Safe ATP-Dependent DNase	1,000 Units	E3101K	Plasmid-Safe ATP Dependent DNase	E0054-10D1	100 µL
			Plasmid-Safe 10X Buffer	SS000272-D8	500 µL
			ATP Solution (25 mM)	SS000408-D1	250 µL
	10,000 Units	E3110K	Plasmid-Safe ATP Dependent DNase (10 U/µL)	E0054-10D2	1 mL
			10X Plasmid-Safe Buffer	SS000272-D9	5 mL
			ATP Solution (25 mM)	SS000408-D2	2 mL

3. 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.

4. 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. Re-suspend the DNA in the appropriate amount of sterile water and set up the Plasmid-Safe DNase reaction as indicated.

For mini-preparations:

x	µL DNA in sterile water
y	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:

x	µL DNA in sterile water
y	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:

x	µL DNA in sterile water
y	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.

5. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com

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