



Product Information

DEOXYRIBONUCLEASE I Amplification Grade

Product Code **AMP-D1**

TECHNICAL BULLETIN

Synonym: DNase I

Product Description

Deoxyribonuclease I (DNase I) is an endonuclease isolated from bovine pancreas that digests double and single stranded DNA into oligo and mononucleotides. Amplification Grade DNase I has been purified to remove RNase activity, and is suitable for eliminating DNA from RNA preparations prior to sensitive applications, such as RT-PCR[†] (Reverse Transcriptase – Polymerase Chain Reaction). No current RNA isolation procedure removes 100% of the DNA. Because PCR can detect even a single molecule of DNA, RNA samples should be digested with DNase I before RT-PCR, and parallel reactions should be run without adding reverse transcriptase to check for amplification of contaminating DNA. These precautions are especially recommended if PCR primers do not span an intron, if pseudogenes that lack the intron may be present in the target cells or tissue,¹ or if the RNA will be used in quantitative RT-PCR.²

Using the Reaction Buffer provided with the Amplification Grade DNase I, the contaminating DNA can be removed from RNA preparations in a 15 minute digestion at room temperature. The DNase I is inactivated by chelating calcium and magnesium ions with EDTA and by heating.³ Heating also denatures RNA secondary structure, so the RNA can be used directly in reverse transcription.

Reagents Provided	Product No.
Sufficient to treat 1000 µg of RNA	
Amplification Grade DNase I, 1 ml of 1 unit/µl in 50% glycerol, 10 mM Tris-HCl, pH 7.5, 10 mM CaCl ₂ , 10 mM MgCl ₂	D 5307
10x Reaction Buffer, 1 ml of 200 mM Tris-HCl, pH 8.3, 20 mM MgCl ₂	R 6273
Stop Solution, 1 ml of 50 mM EDTA	S 4809

Reagents Required but Not Provided

Water, Molecular Biology Reagent, (Product No. W 4502)

Unit Definition

One unit of Amplification Grade DNase I completely digests 1 µg of plasmid DNA to oligonucleotides in 10 minutes at 37 °C. Activity is assayed in a 50 µl reaction containing 33 mM Tris-acetate, pH 7.8, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM dithiothreitol, 1.0 µg DNA, and 0.15 to 2.5 units of DNase I. This unit is equal to approximately 0.75 Kunitz units.

Quality Control

Ten-fold serial dilutions of total RNA are treated with Amplification Grade DNase I according to the procedure below. The DNase-treated RNA and untreated controls are assayed by RT-PCR. No loss in RT-PCR sensitivity is detected with DNase treatment, indicating that the kit components do not interfere with RT-PCR and that they are free from significant RNase activity. No PCR product is detected with 1 µg RNA when reverse transcriptase is omitted from RT reactions, indicating that amplifiable DNA is eliminated.

Storage

The product ships on wet ice and storage at –20 °C in a non-frost free freezer is recommended.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household or other uses. Consult the MSDS for information regarding hazards and safe handling practices.

Procedure

Note that DNase I is sensitive to physical denaturing. Therefore, **do not vortex the Amplification Grade DNase** or the DNase/buffer/RNA mixture before digestion is completed (step 3). Mix by gently flicking the tube or pipetting, and spin briefly to collect the liquid.

Preparation of RNA for RT-PCR

1. Add to an RNase-free PCR tube:
 RNA in 8 µl water
 1 µl of 10x Reaction Buffer
 1 µl of Amplification Grade DNase I, 1 unit/µl

Prepare duplicate tubes for reactions with and without reverse transcriptase. Alternatively, double all volumes and split the digested and denatured product of step 5 into equal portions before adding RT-PCR reagents in step 6.

2. Mix gently, and incubate for 15 minutes at room temperature.
3. Add 1 µl of Stop Solution to bind calcium and magnesium ions and to inactivate the DNase I.

Note: The Stop Solution (50 mM EDTA) **must** be added before heating to prevent metal (Mg/Ca) ion catalyzed hydrolysis of the RNA.

4. Heat at 70 °C for 10 minutes to denature both the DNase I and the RNA.
5. Chill on ice.
6. Add reagents for reverse transcription (RT buffer, primer, dNTPs, RNase inhibitor and reverse transcriptase) or RT-PCR directly to the DNase-treated RNA. Proceed with reverse transcriptase reaction or RT-PCR.

Note: If AMP-D1 is being used with GenElute Mammalian Total RNA kit (Product Code RTN-10, RTN-20, or RTN-350), the entire volume of purified RNA in elution solution may be treated by scaling up the above procedure. For example, RNA in 50 µl of elution solution can be treated by adding 5 µl of 10x Reaction Buffer and 5 µl of Amplification Grade DNase I, and incubating at room temperature for 15 minutes. Inactivate the DNase by adding 5 µl of the Stop Solution and heating at 70 °C for 10 minutes.

References

1. Mutimer, H., et al., Pitfalls of processed pseudogenes in RT-PCR. *BioTechniques*, **24**, 585-588, (1998).
2. Huang, Z., et al., Optimization of Dnase I removal of contaminating DNA from RNA for use in quantitative RNA-PCR. *BioTechniques*, **20**, 1012-1020, (1996).
3. Sanyal, A., et al., An effective method of completely removing contaminating genomic DNA from an RNA sample to be used for PCR. *Mol. Biotechnol.*, **8**, 135-137, (1997).

Related Products	Product No.
GenElute™ Mammalian Total RNA Kits	RTN10, RTN70, or RTN350
GenElute™ Total RNA Kits for Yeast	YTR1-1kit, YTR7-1kit
GenElute™ Total RNA Kits for Bacteria	BTR1-1kit, BTR7-1kit
GenElute™ Direct mRNA Miniprep Kits	DMN10 and DMN70
GenElute™ mRNA Miniprep Kits	MRN10 and MRN70
Tri Reagent	T 9424
Water	W 4502
Enhanced Avian RT-PCR Kits	RT-PCR-20 and RT-PCR-100
qRT-PCR Kits	QR-0100 and QR-0500
Enhanced Avian Reverse Transcriptase	A 4464
Deoxynucleotide (dNTP) Mix	D 7295
Taq DNA Polymerase	D 1806 and D 4545
JumpStart™ Readymix™ REDTaq™	P 0982

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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