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Product Information

DNase I
Amplification Grade

Catalog Number **AMPD1**

TECHNICAL BULLETIN

Synonym: Deoxyribonuclease I

Product Description

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 Sufficient to treat 1000 µg of RNA	**Catalog Number**
DNase I, Amplification Grade 1 ml of 1000 units 1 unit/µL in 50% glycerol, 10 mM Tris-HCl, pH 7.5, 10 mM $CaCl_2$, 10 mM $MgCl_2$	D5307
10x Reaction Buffer, 1 ml of 200 mM Tris-HCl, pH 8.3, 20 mM $MgCl_2$	R6273
Stop Solution, 1 ml of 50 mM EDTA	S4809

Reagents Required but Not Provided

Water, Molecular Biology Reagent, Catalog Number W4502

Unit Definition

One unit of DNase I, Amplification Grade, 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 0.15-2.5 units of DNase I. This unit is equal to ~0.75 Kunitz units.

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 DNase, Amplification Grade, 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 DNase I, Amplification Grade, 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 AMPD1 is being used with GenElute Mammalian Total RNA kit, Catalog Number 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 DNase I, Amplification Grade, 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 Miniprep Kits	RTN10, RTN70, andr RTN350
GenElute Direct mRNA Miniprep Kits	DMN10 and DMN70
GenElute mRNA Miniprep Kits	MRN10 and MRN70
TRI Reagent®	T9424
Water, Molecular Biology Reagent	W 4502
SYBR® Green Quantitative RT-PCR Kit	QR0100
Enhanced Avian Reverse Transcriptase [eAMV™ RT]	A4464
Deoxynucleotide (dNTP) Mix	D7295
Taq DNA Polymerase	D1806 and D4545
JumpStart™ REDTaq® ReadyMix™ Reaction Mix	P0982

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