DNASE I, STERILE LYOPHILIZED

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-NUC-009
Product Name	DNase I, Sterile Lyophilized
Category	Endonuclease
Pack Size	5KU/tube
Regulatory Status	For Research Use Only (RUO)
OEM Reference	GPE017001
Version	1.0
Issue Date	2026-05-14

2. INTENDED USE

DNase I, Sterile Lyophilized is a recombinant RNase-free endonuclease that efficiently removes contaminating DNA from RNA preparations for downstream molecular biology applications including RT-PCR, qPCR, RNA-Seq, and in vitro transcription cleanup. The enzyme cleaves phosphodiester bonds in both single-stranded and double-stranded DNA to generate mono- and oligonucleotides with 5′-phosphate and 3′-hydroxyl termini. Also suitable for reducing viscosity in protein extracts, DNase footprinting, nick translation, and on-column DNA digestion workflows requiring high RNA integrity. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
DNase I, Recombinant (Sterile, Lyophilized)	5,000 U (1 tube)	−20°C
10× DNase I Reaction Buffer	1 mL	−20°C
25 mM EDTA Stop Solution	1 mL	Room temperature (15–25°C)
Nuclease-Free Water	2 mL	Room temperature (15–25°C)

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Materials Required But Not Provided:
- Reaction buffer containing 20 mM Tris-HCl pH 7.5–8.0 and 1–10 mM $MgCl_2$
- Calcium chloride ($CaCl_2$) solution for activity enhancement (optional)
- EDTA solution (0.5 M, pH 8.0) or EGTA for reaction termination
- Nuclease-free water for enzyme reconstitution and dilution
- RNA samples or DNA substrates for treatment
- Appropriate tubes and pipettes for handling enzyme solutions
- Thermal cycler, heat block, or water bath capable of maintaining 37°C
- Equipment for downstream applications (RT-PCR, qPCR, spectrophotometer, gel electrophoresis system)

5. STORAGE AND STABILITY

Storage Temperature	–20°C
Appearance	White or off-white lyophilized powder
Shelf Life	12 months from manufacture date
Shipping Conditions	On dry ice
Freeze-Thaw Cycles	Maximum 3 cycles recommended
Working Solution	Stable on ice for up to 8 hours

6. PRECAUTIONS AND WARNINGS

- For Research Use Only. Not for use in diagnostic procedures.
- Avoid repeated freeze-thaw cycles. Aliquot reagents if needed.
- Handle all reagents on ice. Return to -20°C storage immediately after use.
- Wear appropriate PPE: gloves, lab coat, and eye protection at all times.
- Dispose of waste in accordance with local, state, and federal regulations.
- Do not use reagents past their expiry date.

7. PROTOCOL

DNase I, Sterile Lyophilized

Detailed Application Protocol

ENZOVERA LIFE SCIENCES

For Research Use Only

PRODUCT OVERVIEW

DNase I (Deoxyribonuclease I, E.C. 3.1.21.1) is a recombinant endonuclease that catalyzes the hydrolysis of phosphodiester bonds in both single-stranded and double-stranded DNA, producing mono- and oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. This recombinant bovine pancreatic DNase I is expressed in Pichia pastoris (yeast expression system) and is specifically optimized for molecular biology applications requiring RNase-free DNA removal. The enzyme is supplied as a sterile lyophilized powder with activity greater than or equal to 2000 U/vial (standard) or 5000 U/vial (high activity formulation, lot dependent).

Molecular Weight: 29.89 kDa

Purity: Greater than or equal to 90% by SDS-PAGE

Specific Activity: Greater than or equal to 2000 U/vial or greater than or equal to 5000 U/vial

CAS Number: 9003-98-9

Isoelectric Point (pI): 6.04

Appearance: White or off-white powder

ENZYME CHARACTERISTICS AND MECHANISM

DNase I is a glycoprotein endonuclease requiring divalent cations for catalytic activity. The enzyme exhibits optimal activity at pH 7.8 in the presence of Mg2+ ions, with enhanced activity in the presence of Ca2+. Activity is completely inhibited by chelating agents such as EDTA, EGTA, and citrate buffers. The recombinant expression system eliminates animal-derived pathogen risk and ensures superior lot-to-lot reproducibility compared to tissue-derived preparations.

The enzyme cleaves DNA in a relatively non-specific manner, with slight preference for regions adjacent to pyrimidine nucleotides. Under standard reaction conditions (pH 7–8, 37°C, Mg2+ present), DNase I

produces predominantly oligonucleotides of 2–6 bases in length. Complete digestion to mononucleotides requires extended incubation times and optimized buffer conditions.

APPLICATIONS

1. Removal of genomic DNA contamination from RNA preparations for RT-PCR, qPCR, RNA-Seq, Northern blotting, and microarray analysis

2. On-column DNA digestion during RNA purification workflows

3. In vitro transcription reaction cleanup to eliminate DNA template

4. Reduction of viscosity in protein lysates containing high concentrations of chromosomal DNA

5. DNase I footprinting for protein-DNA interaction mapping

6. Nick translation reactions for probe labeling

7. Chromatin immunoprecipitation (ChIP) sample preparation

8. Single-cell RNA sequencing (scRNA-Seq) sample preparation

STORAGE AND STABILITY

Store lyophilized powder at −20°C in a manual defrost freezer. Under these conditions, the enzyme remains stable for 24 months from the date of manufacture. Avoid repeated freeze-thaw cycles.

Once reconstituted, prepare working aliquots and store at −20°C for up to 6 months. For routine use, working stocks may be stored at 4°C for up to 2 weeks. Do not store reconstituted enzyme in buffers containing chelating agents (EDTA, EGTA) as these will inactivate the enzyme.

RECONSTITUTION OF LYOPHILIZED DNase I

1. Briefly centrifuge the vial at 1000 x g for 10 seconds to collect lyophilized powder at the bottom of the tube.

2. Add sterile nuclease-free water or Reconstitution Buffer (20 mM Tris-HCl pH 7.5, 1 mM MgCl2, 50% v/v glycerol) to achieve the desired final enzyme concentration. For standard reconstitution, add volume to achieve 1 U/microliter final concentration based on the total activity stated on the Certificate of Analysis.

3. Gently mix by pipetting up and down 5–10 times or by gentle vortexing for 5 seconds. Do not vortex vigorously as this may denature the glycoprotein.

4. Allow the solution to stand at room temperature (20–25°C) for 5 minutes to ensure complete dissolution.

5. Centrifuge briefly at 1000 x g for 5 seconds to collect any droplets on the tube walls.

6. Prepare working aliquots (10–50 microliter volumes) and store at −20°C. Avoid repeated freeze-thaw cycles by using single-use aliquots whenever possible.

PROTOCOL 1: DNA REMOVAL FROM RNA PREPARATIONS (IN-SOLUTION DIGESTION)

This protocol removes contaminating genomic DNA from purified total RNA or mRNA samples prior to downstream applications such as RT-PCR, qPCR, or RNA-Seq library preparation.

1. Prepare DNase I Reaction Buffer (10X stock): 200 mM Tris-HCl pH 7.8, 20 mM MgCl2, 2 mM CaCl2. Sterile filter through a 0.22 micron membrane and store at −20°C in single-use aliquots.

2. Thaw RNA sample on ice. Determine RNA concentration by spectrophotometry (A260/A280 ratio should be 1.8–2.1 for pure RNA).

3. In a sterile nuclease-free microcentrifuge tube, assemble the following reaction mixture on ice:

RNA sample: up to 10 micrograms in 10–40 microliters

DNase I Reaction Buffer (10X): 5 microliters

Reconstituted DNase I (1 U/microl

8. EXPECTED RESULTS

Expected Results

When DNase I is used according to the protocol, RNA samples should exhibit no detectable genomic DNA contamination as confirmed by qPCR (Ct values >35 in no-RT controls) or agarose gel electrophoresis showing absence of high-molecular-weight DNA bands. The enzyme should completely digest 1 µg of plasmid DNA within 10 minutes at 37°C in the presence of Mg^{2+}, yielding predominantly mononucleotides and small oligonucleotides with 5′-phosphate termini as verified by denaturing PAGE or capillary electrophoresis. Treated RNA samples should maintain integrity (RIN ≥8.0) and be suitable for downstream applications including RT-PCR, qPCR, RNA-Seq, and in vitro transcription without detectable RNase activity or nucleotide bias.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

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