

# DNase I, RNase-Free

Cat# ER1001 – 1000 U

Storage at -20 °C and avoid from frequent temperature changes

## INTRODUCTION

**DNase I** is an endonuclease that degrades both double-stranded and single-stranded DNA, producing 3'-OH oligonucleotides. DNase I is suited for applications such as nick translation, production of random fragments, cleavage of genomic DNA for footprinting, removal of DNA template after *in vitro* transcription, and removal of DNA from RNA samples prior to applications such as RT-PCR. Moreover, RNase-Free DNase I may be used in applications where maintaining the integrity of the RNA is critical. In the presence of  $Mg^{2+}$ , DNase I attacks each strand of DNA independently, and the sites of cleavage are distributed in a statistically random fashion. In the presence of  $Mn^{2+}$ , DNase I cleaves both strands of DNA at approximately the same site to yield fragments with blunt ends or protruding termini of one or two nucleotides in length.

## CONTENTS

No	Component	ER1001 – 1000U
DA	DNase I, RNase-free (Lyophilized)	1 vial
DB	1X Storage Buffer <sup>a</sup>	1.2 ml
DC	10X Reaction Buffer <sup>b</sup>	1 ml
DD	8X Stop Solution <sup>c</sup>	1 ml

- 1X Storage Buffer: 10 mM HEPES (pH 7.5), 50% glycerol (v/v), 10 mM  $CaCl_2$  and 10 mM  $MgCl_2$ .
- 10× Reaction Buffer: 400 mM Tris-HCl (pH 8.0), 100 mM  $MgSO_4$  and 10 mM  $CaCl_2$ .
- 8X Stop Solution: 20 mM EDTA (pH 8.0).

## INFORMATION

**Inhibitors:** EGTA; EDTA; salt concentrations >100mM will reduce DNase activity.

**Molecular Weight:** 31,000 Daltons.

**Requirement:**  $Ca^{2+}$  and  $Mg^{2+}$  or  $Mn^{2+}$ .

**Source:** Bovine pancreas.

## UNIT DEFINITION

One unit of RNase-Free DNase is defined as the amount required to completely degrade 1  $\mu$ g of lambda DNA in 10 minutes at 37°C in 50 $\mu$ l of a buffer containing 40mM Tris-HCl (pH 7.9), 10mM NaCl, 6mM  $MgCl_2$  and 10mM  $CaCl_2$ . Under these assay conditions one unit of DNase activity is approximately equal to one Kunitz unit.

## **QUALITY CONTROL**

RNase Assay: 50ng of [<sup>3</sup>H]RNA is incubated with 5 units of RNase-Free DNase I in Transcription Optimized 1x Buffer for 1 hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. The minimum passing specification is <3% release.

## **PROTOCOL**

**Note** : This DNase solution does not contain an RNase inhibitor. Observe caution in handling the product to ensure against contaminating it with RNase.

### **1. Preparation**

Resuspend DNase I in 1 ml Storage Buffer and aliquot into appropriated volume, store at -20°C .

### **2. Removal of genomic DNA from RNA**

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Mix the component in RNase-free tube

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DNase I, RNase-free (1 U/1 µl)	1 µl
10X Reaction Buffer	5 µl
RNA	Optional
RNase-free ddH <sub>2</sub> O	To 50 µl

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- 3.** Mix thoroughly and incubate at 37°C for 10 mins.
- 4.** Add Stop solution to a final 2.5 mM.
- 5.** Heat inactivate at 65°C for 10 mins.

## **PRODUCT USE LIMITATION**

These products are intended for research use only.