For life science research only. Not for use in diagnostic procedures.

DNase I recombinant, RNase-free

From bovine pancreas, expressed in *Pichia pastoris*
Enzyme solution with 10× Incubation Buffer
Deoxyribonuclease I, EC 3.1.21.1
Cat. No. 04 716 728 001
10 000 units

Version 3.0
Content version: June 2008

Store at −15 to −25°C

1. What this product does

Form

Solution, 10 units/µl

Storage and Stability

When stored at −15 to −25°C, the enzyme is stable through the expiration date printed on the label.

Storage Buffer

20 mM Tris-HCl, 50 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM dithioerythritol, 0.1 mg/ml Pefabloc SC*, 50% glycerol (v/v), pH 7.6 (at +4°C)

Incubation Buffer (10×)

400 mM Tris-HCl, 100 mM NaCl, 60 mM $MgCl_2$, 10 mM $CaCl_2$, pH 7.9, 5 vials, 1 ml each

Application

DNase I recombinant, RNase-free may be used to degrade DNA in applications that are sensitive to the presence of RNase.

For example, DNase I is frequently used to

- remove genomic DNA from RNA preparations prior to RT-PCR
- isolate DNA-free RNA after *in vitro* transcription reactions (3)
- perform nick translations (4)
- map DNase-sensitive regions in eukaryotic DNA (5).

2. How to use this product

2.1 Complete digestion on DNA

- Prepare the following mixture

Component	Final concentration
DNA	1 µg
10× Incubation Buffer	2 µl
DNase I recombinant, RNase-free	1 – 2 U
Water, RNase-free	up to 20 µl

- Incubate at +25 to +37°C for 10 min.
- Use the sample for further analysis.

2.2 Digestion of Genomic DNA in RNA Samples

- Prepare the following mixture

Component	Final concentration
Total RNA	10 - 50 µg
10x Incubation Buffer	5 µl
DNase I recombinant, RNase-free	2.5 - 10 units
Optionally: Protector RNase Inhibitor*	10 units
Water, RNase-free	up to 50 µl

- Incubate at +25 to +37°C for 15 - 20 min.
- Stop the reaction by adding 2 µl of 0.2 M EDTA (pH 8.0) to a final concentration of 8 mM and heating to +75 °C for 10 min (6). The concentration of EDTA has to be taken into account for all subsequent applications.

2.3 Inactivation

1 unit DNase I recombinant, RNase-free is heat-inactivated by 10 min incubation at 75 °C (7).

⚠ Alternatively, DNase I recombinant, RNase-free can be inactivated and removed by phenol extraction according to standard protocols, *e.g.,* Current Protocols in Molecular Biology (8).

3. Additional Information on this Product

3.1 How this Product Works

DNase I recombinant, RNase-free is a recombinant form of DNase I from bovine pancreas, expressed in *Pichia pastoris*.

It is a glycoprotein of a molecular weight of ~ 39 kD. The recombinant enzyme is produced without using any animal cells or other materials derived from animals.

DNase I is a DNA-specific endonuclease that hydrolyses the phosphodiester linkages of double-stranded or single-stranded DNA to a mixture of oligo- and mononucleotides (1). The enzyme requires divalent cations for maximal activity (2).

Activity

10×10^3 units/ml.

One unit is the enzyme activity that effect an absorbance increase of 0.001/min under assay conditions in 1 ml at 260 nm.

Assay Conditions

Volume activity is determined according to the following assay mixture. 100 µg calf thymus DNA are incubated in 2.5 ml 1× incubation buffer with 40 – 70 units DNase I recombinant, RNase-free at +25°C. The absorbance increase is measured at 260 nm

Enzyme Dilution buffer

25 mM Tris-HCl, 50% glycerol (v/v), pH 7.6 (at +4°C).

3.2 References

1 Kunitz, M. (1950) *J. Gen. Physiol.* **33,** 349 – 362
2 Eichhorn, G.L. et al. (1969) *J. Biol. Chem.* **244,** 937-942
3 Krieg, P. A. et al. (1985) in Genetic Engineering Principles and Methods(Setlow, J. K., Hollaender, A., ed.) Vol. 7, Plenum Press, New York, London.
4 Rigby, P. W. et al. (1977) *J. Mol. Biol.* **113,** 237.
5 Weisbrod, S. (1982) *Nature* **297**, 289.
6 Wiame, I. et al. (2000) *BioTechniques* **29,** 252-259.
7 Huang, Z et al (1996) *BioTechniques* **20,** 1012-1020.
8 Ausubel, F.M. et al. (Eds) Current Protocols in Molecular Biology (2004), Wiley & Sons; Four Volumes 0-741-50338-X-Losseleaf or 0-471-30661-4-CD-ROM.

3.3 Quality Control

Absences of RNases

5 µg of MS2-RNA are incubated with 50 units of DNase I recombinant, RNase-free for 4 h at +37° C in 50 µl 25 mM Tris-HCl, 5 mM $MgCl_2$, 0.01 mM EDTA, pH 7.6. No degradation of MS2-RNA is observed.

Absence of Proteases

200 µg of resorufin-labeled casein is incubated with 50 units DNase I recombinant, RNase-free for 17 h at +37°C in 40 mM Tris-HCl, 4 mM $CaCl_2$, pH 7.8. The reaction is stopped by the addition of trichloroacetic acid (TCA) and then centrifugated. The pH of the supernatant is adjusted with 500 mM Tris-HCl, pH 8.8. The absorbance is then measured at 574 nm to determine if the supernatant contains any resorufin-labeled peptides that were not precipitated by TCA. In this assay, 50 U DNase I does not produce a detectable signal at 574 nm.

4. Supplementary Information

4.1 Conventions

Text Conventions

To make information consistent and memorable, the following text convention are used in this package insert:

Text Conventions	Use
Asterisk*	Denotes a product available from Roche Applied Science

Symbols

In this package insert the following symbol is used to highlight important information

Symbol	Description
⚠	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Ordering Information

For a complete overview of related products, please visit and bookmark our Special Interest Sites for:

- manual Nucleic Acid Isolation and Purification: http://www.roche-applied-science.com/napure/

Also visit the Roche Applied Science site to find the following Printed Materials:

- Nucleic Acid Isolation and Purification Manual
- NAPI Product Selection Guide

Product	Pack size	Cat. No.
Protector RNase Inhibitor	2 000 U 10 000 U (5 vials of 2000 U)	03 335 399 001 03 335 402 001
DIG RNA Labeling Kit (SP6/T7)	1 kit (2× 10 labeling	11 175 025 910
DIG Northern Starter Kit	10 labeling/detection reactions	12 039 672 910
High Pure RNA Isolation Kit	1 kit (50 reactions)	11 828 665 001
High Pure RNA Paraffin Kit	1 kit (100 isolations)	03 270 289 001
High Pure RNA Tissue Kit	1 kit (50 isolations)	12 033 674 001
Transcriptor Reverse Transcriptase	2000 U (4× 500 U) for 200 reactions 500 U for 50 reactions 250 U for 25 reactions	03 531 295 001 03 531 295 001 03 531 317 001
Transcriptor First Strand cDNA Synthesis Kit	50 reactions	04 379 012 001
DNA Polymerase I, endonuclease-free	250 units 1000 units	10 642 711 001 10 642 720 001
Pefabloc SC	100 mg 500 mg 1 g	11 429 868 001 11 585 916 001 11 429 876 001
cOmplete Protease Inhibitor Cocktail Tablets	20 tablets 3 × 20 tablets	11 697 498 001 11 836 145 001
Tris-HCl	500 g	10 812 846 001

4.3 Trademarks

Regulatory Disclaimer:

For life science research only. Not for use in diagnostic procedures.

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