

CERTIFICATE OF ANALYSIS

**Deoxyribonuclease I (DNase I),
RNase-free**

#EN0525 1000u (1u/μl)

Lot: **Expiry Date:**

Concentration: 1u/μl

Supplied with: 1ml of 10X Reaction Buffer with MgCl₂
 1ml of 10X Reaction Buffer without MnCl₂
 1ml of 100mM MnCl₂
 1ml of 25mM EDTA

Store at -20°C

In total 5 vials.

Description

Deoxyribonuclease I (DNase I) is an endonuclease that digests single- and double-stranded DNA. It hydrolyzes phosphodiester bonds producing mono- and oligodeoxyribonucleotides with 5'-phosphate and 3'-hydroxyl groups. The enzyme activity is strictly dependent on Ca²⁺ and is activated by Mg²⁺ and Mn²⁺ ions:

- in the presence of Mg²⁺, DNase I cleaves each strand of dsDNA independently in a statistically random fashion (1);
- in the presence of Mn²⁺, the enzyme cleaves both DNA strands at approximately the same site, producing DNA fragments with blunt-ends or with protruding termini of only one or two nucleotides (1).

Source

Bovine pancreas.

Molecular Weight

32kDa monomer.

Definition of Activity Unit

One unit of the enzyme completely degrades 1μg of plasmid DNA in 10min at 37°C.

Enzyme activity is assayed in the following mixture:
40mM Tris-HCl (pH 8.0), 10mM MgSO₄, 1mM CaCl₂,
1μg of pBR322 DNA.

Storage Buffer

The enzyme is supplied in: 50mM Tris-acetate (pH 7.5),
10mM CaCl₂ and 50% (v/v) glycerol.

10X Reaction Buffer with MgCl₂

100mM Tris-HCl (pH 7.5 at 25°C), 25mM MgCl₂, 1mM CaCl₂.

10X Reaction Buffer without MnCl₂

100mM Tris-HCl (pH 7.5 at 25°C), 1mM CaCl₂. Recommended concentration of MnCl₂ in 1X reaction buffer is 10mM.

Applications

- Preparation of DNA-free RNA (1).
- Removal of template DNA following *in vitro* synthesis of RNA with T7, T3, SP6 RNA Polymerases (1), see protocol on back page.
- Preparation of DNA-free RNA prior to RT-PCR (2), see protocol on back page.
- DNA labeling by nick-translation in conjunction with DNA Polymerase I (1), see protocol on back page.
- Studies of DNA-protein interactions by Deoxyribonuclease I footprinting (1).
- Generation of a library of randomly overlapping DNA inserts. Mn²⁺ is required in the reaction buffer (3), see Related Products.

Inhibition and Inactivation

- Inhibitors: metal chelators, transition metals (e.g., Zn) in millimolar concentrations, SDS (even at concentrations less than 0.1%), reducing agents (DTT and β-mercaptoethanol), ionic strength above 50-100mM.
- Inactivated by heating at 65°C for 10min in the presence of EGTA or EDTA or by phenol/chloroform extraction.


QUALITY CONTROL ASSAY DATA

Ribonuclease Assay

0% of the total radioactivity was released into the trichloroacetic acid-soluble fraction after incubation of 10 units of Deoxyribonuclease I with 1μg of [³H]-RNA in 50μl of reaction buffer with MgCl₂ for 4 hours at 37°C.

Functional Assay

Deoxyribonuclease I was tested for digestion of template DNA following *in vitro* synthesis of RNA using T7 phage RNA Polymerase.

Quality authorized by:  Jurgita Zilinskiene

(for protocols see back page)

Protocol for Removal of Template DNA Following *in vitro* Synthesis of RNA

- 1 Add 2u of Deoxyribonuclease I (DNase I), RNase-free, per 1µg of template DNA directly to a transcription reaction mixture. In some cases, the amount of enzyme required to completely digest the DNA should be determined empirically.
- 2 Incubate at 37°C for 15 minutes.
- 3 Inactivate DNase I by phenol/chloroform extraction.

Protocol for Preparation of DNA-free RNA prior to RT-PCR

- 1 Add to an RNase-free tube:

RNA	1µg
10X reaction buffer with MgCl ₂ ,	1µl
DEPC-treated Water (#R0601)	to 9µl
Deoxyribonuclease I (DNase I), RNase-free (1u/µl)	1µl (1u)

Note

If larger amounts of DNA-free RNA are required, the reaction mixture should be scaled up. Ribonuclease inhibitor (typically 1u/µl) can also be included in the reaction mixture to prevent RNA degradation.

- 2 Incubate at 37°C for 30 minutes.
- 3 Add 1µl 25mM EDTA and incubate at 65°C for 10 minutes. RNA hydrolyzes during heating in the absence of a chelating agent (4).
- 4 Use the prepared RNA as a template for reverse transcriptase.

Protocol for DNA Labeling by Nick-translation

- 1 Mix the following components:

10X reaction buffer for DNA Polymerase I	2.5µl
mixture of 3 dNTPs, 1mM* (without the labeled dNTP)	1.25µl
[α- ³² P]-dNTP, ~110TBq/mmol (3000Ci/mmol)	1.85-3.7MBq (50-100µCi)
Deoxyribonuclease I (DNase I), RNase-free freshly diluted to 0.002u/µl**	1µl
DNA Polymerase I, <i>E.coli</i> (#EP0041)	0.5-1.5µl (5-15u)
template DNA	0.25µg
Water, nuclease-free (#R0581)	to 25µl

- 2 Immediately incubate at 15°C for 15 to 60 minutes.
- 3 Terminate the reaction by adding 1µl of 0.5M EDTA, pH 8.0 (#R1021).
- 4 Take an aliquot (1µl) to determine the efficiency of label incorporation. A specific activity of at least 10⁸cpm/µg DNA is expected.
- 5 If required, the labeled DNA may be separated from the unincorporated radioactive precursors on Sephadex G-50 or Bio-Gel P-60 column.

Note

* To prepare a mixture of 3 non-labeled dNTPs (1mM of each), mix 1µl aliquots of stock solutions of each dNTP (100mM, from #R0181) with 97µl of Water, nuclease-free (#R0581). These dNTP mixes can be stored at -20°C for later use.

** Deoxyribonuclease I (DNase I), RNase-free can be diluted with the 1X reaction buffer for DNA Polymerase I.

- 10X Reaction Buffer for DNA Polymerase I: 500mM Tris-HCl (pH 7.5 at 25°C), 100mM MgCl₂ and 10mM DTT.
- The reaction volumes can be scaled up or down as long as the final concentrations of the components (DNA, dNTPs, labeled dNTP) remain as indicated in the protocol.
- Radioactive DNA probes with higher specific activities can be prepared using two radioactively labeled dNTPs simultaneously. In this case, the composition of the unlabeled dNTP mix should be adjusted accordingly.

References

1. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
2. Kienzle, N., et al., DNase I treatment is a prerequisite for the amplification of cDNA from episomal-based genes, *BioTechniques*, 20, 612-616, 1996.
3. Anderson, S., Shotgun DNA sequencing using cloned DNase I-generated fragments, *Nucleic Acids Res.*, 9, 3015-3027, 1981.
4. Wiame, I., et al., Irreversible heat inactivation of DNase I without RNA degradation, *BioTechniques*, 29, 252-256, 2000.

Related Products

- SP6 RNA Polymerase #EP0131, #EP0133
- T3 RNA Polymerase #EP0101, #EP0102, #EP0103
- T7 RNA Polymerase #EP0111, #EP0112, #EP0113
- RevertAid™ M-MuLV Reverse Transcriptase (*not available in the USA*) #EP0441, #EP0442
- RevertAid™ H Minus M-MuLV Reverse Transcriptase (*not available in the USA*) #EP0451, #EP0452
- M-MuLV Reverse Transcriptase #EP0441, #EP0442
- DNA Polymerase I, *E.coli* #EP0041, #EP0042
- RiboLock™ Ribonuclease Inhibitor #EO0381, #EO0382
- Ribonuclease Inhibitor (from human placenta) #EO0311, #EO0312
- First Strand cDNA Synthesis Kit #K1611, #K1612
- RevertAid™ First Strand cDNA Synthesis Kit (*not available in the USA*) #K1621, #K1622
- RevertAid™ H Minus First Strand cDNA Synthesis Kit (*not available in the USA*) #K1631, #K1632
- Water, nuclease-free #R0581, #R0582
- DEPC-treated Water #R0601, #R0603

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

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