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IMPROVED recombinant protein

CERTIFICATE OF ANALYSIS

DNase I, RNase-free

#EN0521 1000 u

Lot: **Expiry Date:**

Concentration: 1 u/µl

Supplied with: 1 ml of 10X Reaction Buffer with $MgCl_2$
1 ml of 50 mM EDTA

Store at -20°C

In total 3 vials.

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Description

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'-OH groups.
The enzyme activity is strictly dependent on Ca^{2+} and is activated by Mg^{2+} or Mn^{2+} ions:

- in the presence of Mg^{2+}, DNase I cleaves each strand of dsDNA independently, in a statistically random fashion (1);
- in the presence of Mn^{2+}, the enzyme cleaves both DNA strands at approximately the same site, producing DNA fragments with blunt ends or with one or two nucleotide overhangs (1).

Applications

- Preparation of DNA-free RNA (1).
- Removal of template DNA following *in vitro* transcription (1), *see* protocol on reverse page.
- Preparation of DNA-free RNA prior to RT-PCR and RT-qPCR (2), *see* protocol on reverse page.
- DNA labeling by nick-translation in conjunction with DNA Polymerase I (1), *see* protocol on reverse page.
- Studies of DNA-protein interactions by DNase I, RNase-free footprinting (1).
- Generation of a library of randomly overlapping DNA inserts. Reaction buffer containing Mn^{2+} is used (3).

Source

E.coli cells with a cloned gene encoding bovine DNase I.

Molecular Weight

29 kDa monomer.

Definition of Activity Unit

One unit of the enzyme completely degrades 1 µg of plasmid DNA in 10 min at 37°C.
Enzyme activity is assayed in the following mixture: 10 mM Tris-HCl (pH 7.5 at 25°C), 2.5 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 µg of pUC19 DNA. One DNase I unit is equivalent to 0.3 Kunitz unit (4).

Storage Buffer

The enzyme is supplied in: 50 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$ and 50% (v/v) glycerol.

10X Reaction Buffer with $MgCl_2$

100 mM Tris-HCl (pH 7.5 at 25°C), 25 mM $MgCl_2$, 1 mM $CaCl_2$.

Inhibition and Inactivation

- Inhibitors: metal chelators, transition metals (e.g., Zn) in millimolar concentrations, SDS (even at concentrations less than 0.1%), reducting agents (DTT and β-mercaptoethanol), ionic strength above 50-100 mM.
- Inactivated by heating at 65°C for 10 min in the presence of EGTA or EDTA (use at least 1 mol of EGTA/EDTA per 1 mol of Mn^{2+}/Mg^{2+} (5)).

Note

DNase I is sensitive to physical denaturation. Mix gently by inverting the tube. Do not vortex.

QUALITY CONTROL ASSAY DATA

Ribonuclease Assay

No degradation of RNA was observed after incubation of 5 units of DNase I with 160 ng RNA in 20 µl of reaction buffer (10 mM Tris-HCl (pH 7.5 at 25°C), 2.5 mM $MgCl_2$, 0.1 mM $CaCl_2$) for 4 hours at 37°C.

Quality authorized by:  Jurgita Zilinskiene

(continued on reverse page)

Removal of genomic DNA from RNA preparations

1. Add to an RNase-free tube:

RNA	1 µg
10X reaction buffer with $MgCl_2$	1 µl
DNase I, RNase-free (#EN0521)	1 µl (1 u)
DEPC-treated Water (#R0601)	to 10 µl

2. Incubate at 37°C for 30 min.
3. Add 1 µl 50 mM EDTA and incubate at 65°C for 10 min. RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent (5). Alternatively, use phenol/chloroform extraction.
4. Use the prepared RNA as a template for reverse transcriptase.

Note

- Do not use more than 1 u of DNase I, RNase-free per 1 µg of RNA.
- Volumes of the reaction mixture and 50 mM EDTA solution can be scaled up for larger amounts of RNA. The recommended final concentration of RNA is 0.1 µg/µl.
- RiboLock™ RNase Inhibitor (#EO0381), typically at 1 u/µl, can also be included in the reaction mixture to prevent RNA degradation.

Removal of template DNA after *in vitro* transcription

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

DNA labeling by nick-translation

1. Mix the following components:

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

2. Immediately incubate at 15°C for 15-60 min.
3. Terminate the reaction by adding 1 µl of 0.5 M EDTA, pH 8.0 (#R1021).
4. Take an aliquot (1 µl) to determine the efficiency of label incorporation. A specific activity of at least 10^8 cpm/µg DNA is expected.

Note

- The labeled DNA can be purified from the unincorporated labelled dNTPs using GeneJET™ PCR Purification Kit (#K0701).

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

****** DNase I, RNase-free can be diluted with 1X reaction buffer for DNA Polymerase I: 50 mM Tris-HCl (pH 7.5 at 25°C), 10 mM $MgCl_2$ and 1 mM DTT.

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. Kunitz, M., J.Gen.Physiol., 33, 349-362, 1950.
5. Wiame, I., et al., Irreversible heat inactivation of DNase I without RNA degradation, BioTechniques, 29, 252-256, 2000.