DNaseI, RNase- free (500 u)

Concentration:1u/µl **Cat. No.**: PR891627

Supplied with: 1 ml of 10X Reaction Buffer with $MgCl_2$, 1 ml of 50 mM EDTA **Store at -20°C**

Description:

DNaseI is an endonuclease that digests single- and double-stranded DNA. It hydrolyzes phosphodiester bonds producing mono and oligodeoxyribo- nucleotides 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+}, DNaseI cleaves each strand of dsDNA independently, in a statistically random fashion;
- 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 .

Applications:

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

Source
E.coli cells with a cloned gene encoding bovine DNaseI.

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 DNaseI unit is equivalent to 0.3 Kunitz unit.

Storage Buffer
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%), reducing agents (DTT and 2-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+}).

Note

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

Removal of genomic DNA from RNA preparations

1. Add to an RNase-free tube:

RNA	1 µg
10X reaction buffer with MgCl2	1 µl
DNaseI, RNase-free	0.5µl (0.5u)

DEPC-treated Water	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. 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 DNaseI, 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, 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 2u of DNaseI, 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 DNaseI by phenol/chloroform extraction.

DNA labeling by nick-translation

- 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
[32P]-dNTP, ~110 TBq/mmol (3000 Ci/mmol)	1.85-3.7 MBq (50-100 µCi)
DNaseI, RNase-free freshly diluted to 0.002 u/µl	0.5 µl
DNA Polymerase I Template DNA	0.5-1.5 µl (5-15u) 0.25 µg
Water, nuclease-free	to 25 µl

- Immediately incubate at 15°C for 15-60 min.
- Terminate the reaction by adding 1 µl of 0.5 M EDTA, pH 8.0.
- 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

- DNaseI, 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 MgCl2 and 1 mM DTT

Quality Control Assay Data

No degradation of RNA was observed after incubation of 5 units of DNaseI with 160 ng RNA for 4 hours at 37°C.