DNaseI, RNase-free (500 u)

For Research Use Only

Cat. No.:MO5401 **Concentration:** 1u/µl

Supplied with: 1ml of 10X Reaction Buffer with $MgCl_2$,1ml of 50mM 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 byMg^{2+} orMn^{2+} ions:

- In the presence ofMg^{2+}, DNaseI cleaves each strand of ds DNA independently, in a statistically random fashion;
- In the presence ofMn^{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 10min at 37°C.

Enzyme activity is assayed in the following mixture: 10mM Tris-HCl (pH 7.5 at 25°C), 2.5mM $MgCl_2$, 0.1mM $CaCl_2$, 1 µg of pUC19 DNA.

One DNaseI unit is equivalent to 0.3 Kunitz unit.

Storage Buffer

50mM Tris-HCl (pH 7.5), 10mM $CaCl_2$ and 50% (v/v) glycerol.

10X Reaction Buffer with $MgCl_2$

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

Inhibition and Inactivation

- Inhibitors:Metal chelators, transitionMetals (e.g., Zn) in Millimolar concentrations, SDS (even at concentrations less than 0.1%), reducing agents (DTT and 2-Mercaptoethanol), ionic strength above 50-100mM.
- Inactivated by heating at 65°C for 10Min in the presence of EGTA or EDTA (use at least 1mol of EGTA/EDTA per 1mol 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 $MgCl_2$	1µl
DNaseI, RNase-free	0.5µl (0.5u)
DEPC-treated Water	to 10µl

2. Incubate at 37°C for 30min.
3. Add 1µl 50mM EDTA and incubate at 65°C for 10min. 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 50mM 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 15Minutes.
3. Inactivate DNaseI by phenol/chloroform extraction.

DNA labeling by nick-translation

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

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

4. Take an aliquot (1µl) to determine the efficiency of label incorporation. A specific activity of at least 10^8cpm/µg DNA is expected.

Note:

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

Quality Control Assay Data

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

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