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یکتا تجهیز آزما

DNase I , RNase-free for research use only

Cat No: YT8054 **Size: 1000 U** **Concentration: 1u/µl**

components	YT8054
DNase I , RNase free (1u/µl)	1 ml
10x reaction buffer with $MgCl_2$	1 ml
50mM EDTA	1 ml

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 Ca2+ and is activated by Mg2+ or 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 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 Mn2+ is used (3).

Source

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

Molecular Weight : 29 kDa monomer.

Definition of Activity Unit2

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 MgCl2,
0.1 mM CaCl2, 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 CaCl2 and 50% (v/v) glycerol.

10X Reaction Buffer with MgCl2

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

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 50-100 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 Mn2+/Mg2+ (5)).

Removal of genomic DNA from RNA preparations:

1. Add to an RNase-free tube:	
RNA	1 µg
10X reaction buffer with MgCl2	1µL
Dnase I , RNase-free	1µL(1U)
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 (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.
- If using DNase I, HC, enzyme can be diluted in 1X DNase reaction buffer just prior to use, or in storage buffer (not supplied *see composition on reverse page*) for longer storage.
- 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.

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.

Store at -20 ˚C.