DNase I, RNase-Free, 1 U/µL

FB02514100 1000 Units

Store at -25°C to -15°C

DNase I is an endonuclease that digests both single- and double-stranded DNA by hydrolyzing phosphodiester bonds, resulting in 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+} ions. In the presence of Mg^{2+}, DNase I cleaves each strand of dsDNA independently, in a statistically random fashion.

Applications

- Preparation of DNA-free RNA (Protocol 1).
- Removal of template DNA following *in vitro* transcription (Protocol 2).
- DNA labeling by nick-translation in conjunction with DNA Polymerase I.
- Studies of DNA-protein interactions by DNase I, RNase-free footprinting.

Kit Contents

Reagents	Volume
DNase I, RNase-Free, 1 U/µL, 1000 U	1mL
10X Reaction Buffer with $MgCl_2$	1.25 mL
50 mM EDTA	1 mL

Definition of Activity Unit

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

Protocol 1: Removal of genomic DNA from RNA preparations

1. Prepare the following reaction mixture in a RNase-free tube:

Component	10 µL Reaction
RNA	1 µg
10X Reaction Buffer with $MgCl_2$	1 µL
DNase I, RNase-Free, 1 U/µL,	1 µL (1 U)
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 transcription.

Note:

- Do not use more than 1 U of DNase I, RNase-free per 1µg 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.
- RNase Inhibitor (FB02518250) typically at 1 U/µL, can also be included in the reaction mixture to prevent RNA degradation.

Protocol 2: 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.