

## Product Description

Deoxyribonuclease I (DNase I) is an endonuclease that digests single- or double-stranded DNA. It recognizes and cleaves phosphodiester bonds to produce a single deoxynucleotide or single- or double-stranded oligodeoxynucleotide with 5'-phosphorylated and 3'-hydroxylated ends. The activity of DNase I depends on  $\text{Ca}^{2+}$ , and DNase I can also be activated by divalent metal ions such as  $\text{Mg}^{2+}$  and  $\text{Mn}^{2+}$ . In the presence of  $\text{Mg}^{2+}$ , DNase I nonspecifically recognizes and cleaves a double-stranded DNA at any site on either strand, and in the presence of  $\text{Mn}^{2+}$ , it recognizes and cleaves almost the same sites on both strands of the DNA to produce DNA fragments with blunt ends or sticky ends with one or two nucleotide overhangs.

## Components

Components	EN401-01/02	EN402-01/02
	1,000 U/10,000 U	1,000 U/10,000 U
DNase I, RNase-free (1 U/ $\mu\text{l}$ )	1 ml/10 ml	-
DNase I, RNase-free (50 U/ $\mu\text{l}$ )	-	20 $\mu\text{l}$ /200 $\mu\text{l}$
DNase I Dilution Buffer	-	1 ml/10 ml
10 × Reaction Buffer	1 ml/10 ml	1 ml/10 ml

## Storage

Store at  $-30 \sim -15^{\circ}\text{C}$  and transport at  $\leq 0^{\circ}\text{C}$ .

## Applications

It is applicable for RNA extraction, *in vitro* transcription, DNA removal in RT-PCR, DNase I footprinting, nick translation, DNA library preparation and other molecular biological experiments.

## Source

Recombinase, purified from non-animal hosts.

## Unit Definition

One unit (U) is defined as the amount of enzyme which will completely degrade 1  $\mu\text{g}$  of pUC19 plasmid DNA at  $37^{\circ}\text{C}$  in 10 min.

## Notes

For research use only. Not for use in diagnostic procedures.

- When using this product to remove DNA from an RNA sample, Murine RNase inhibitor (Vazyme #R301) can be added to the reaction solution to protect the RNA from degradation.
- The optimal amount of DNase I needs to be adjusted under some experimental conditions.
- Deactivation or inhibition: The DNase I can be deactivated by adding EDTA at a final concentration of 5 mM and heating at  $65^{\circ}\text{C}$  for 10 min, or by phenol/chloroform extraction. Furthermore, chelating agents, a certain concentration of  $\text{Zn}^{2+}$ , 0.1% SDS, reducing agents such as DTT and mercaptoethanol, and salinity levels of more than 50 - 100 mM could significantly inhibit the activity of DNase I.

## Experiment Process

### 1. Removal of DNA from RNA sample before RT-PCR

a. Prepare the following mixture in an RNase-free centrifuge tube:

Components	Volume
RNase-free ddH <sub>2</sub> O	to 10 µl
10 × Reaction Buffer	1 µl
DNase I, RNase-free (1 U/µl)	1 µl*
RNA	X

Thoroughly mix by pipetting up and down gently with a pipette and incubate at 37°C for 15 min.

\* When using EN402 for the experiment, dilute the DNase I with DNase I Dilution Buffer to an appropriate concentration before use.

b. Add EDTA to stop the reaction:

Components	Volume
Mixture from the previous step	10 µl
EDTA (50 mM)	1 µl

Thoroughly mix by pipetting up and down gently with a pipette and incubate at 65°C for 10 min.

c. The processed RNA sample can be used as template for subsequent RT-PCR.

### 2. Removal of template DNA after *in vitro* transcription

a. Add 1 U of DNase I to the transcription reaction system for every 0.5 µg of template DNA.

\* When using EN402 for the experiment, dilute the DNase I with DNase I Dilution Buffer to an appropriate concentration before use.

b. Thoroughly mix by pipetting up and down gently with a pipette and incubate at 37°C for 15 min.

c. Deactivate the DNase I by phenol/chloroform extraction.

