DNase I, RNase-free

EN401/EN402

Version 23.1

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 Ca^{2+}, and DNase I can also be activated by divalent metal ions such as Mg^{2+} and Mn^{2+}. In the presence of Mg^{2+}, DNase I nonspecifically recognizes and cleaves a double-stranded DNA at any site on either strand, and in the presence of 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 1,000 U/10,000 U	EN402-01/02 1,000 U/10,000 U
DNase I, RNase-free (1 U/μl)	1 ml/10 ml	-
DNase I, RNase-free (50 U/μl)	-	20 μl/200 μl
DNase I Dilution Buffer	-	1 ml/10 ml
10 × Reaction Buffer	1 ml/10 ml	1 ml/10 ml

Storage

Store at -30 ~ -15°C and transport at ≤0°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 μg of pUC19 plasmid DNA at 37°C in 10 min.

Notes

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

1. 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.
2. The optimal amount of DNase I needs to be adjusted under some experimental conditions.
3. Deactivation or inhibition: The DNase I can be deactivated by adding EDTA at a final concentration of 5 mM and heating at 65°C for 10 min, or by phenol/chloroform extraction. Furthermore, chelating agents, a certain concentration of 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_2O	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.