DNase I, RNase-free

EN401/402

Version 21.1

Product Description

Deoxyribonuclease I (DNase I), is an endodeoxyribonuclease 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 a phosphate group at the 5'-end and a hydroxyl group at the 3'-end. DNase I depends on Ca^{2+} for activity, and it 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 to -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, preparation of random DNA fragment libraries, and other molecular biological experiments.

Source

Recombinant enzyme derived from non-animal host.

Unit Definition

One active unit (U) is defined as the amount of enzyme required to completely degrade 1 μg of pUC19 plasmid DNA at 37℃ for 10 min.

Quality Control

Purity: ≥90%;

RNase residual test: No residual RNase was detected in 100 U of this product when tested based on the RNaseAlert QC System (Thermo Fisher #AM1966) instruction manual.

Function test: 500 ng of human genomic DNA was added and processed with 1 U of DNase I. qPCR was then performed with two sets of quality control primers, and the removal efficiency was >99.9%.

Notes

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 65ºC for 10 min, or by extraction using phenol chloroform. Furthermore, chelating agents, zinc ions at mM concentration, 0.1% SDS, reducing agents such as DTT and mercaptoethanol, and salinity of at least 50 - 100 mM are all significantly effective in inhibiting the activity of DNase I.

Examples

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

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

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:

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 then be used as template for the 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.