

DNase I (RNase-free)

(M010)

Product Description

DNase I (Deoxyribonuclease I) is a deoxyribonuclease that digests single-stranded or double-stranded DNA. It cleaves phosphodiester bonds to produce mono-deoxynucleotides or single/double-stranded oligodeoxynucleotides with **5'-phosphate and 3'-hydroxyl termini**.

DNase I activity is **Ca²⁺-dependent** and can be activated by divalent metal ions such as Mg²⁺ and Mn²⁺. In the presence of Mg²⁺, the enzyme randomly cleaves either strand of double-stranded DNA. In the presence of Mn²⁺, it cleaves both DNA strands at nearly identical sites, generating blunt-ended DNA fragments or sticky-ended fragments with 1–2 nucleotide overhangs.

Component

Cat. No.	Product Name	Volume
M010-A	DNase I (RNase-free)	2 U/μL, 1 KU
M010-B	10 x DNase I Buffer	1.5 mL

Definition of Activity

One unit (U) is defined as the amount of enzyme required to **completely degrade 1 μg of pUC19 plasmid DNA** in 10 minutes at 37 °C.

Applications

Suitable for removing DNA in RNA extraction, in vitro transcription, and RT-PCR; DNase I footprinting; nick translation; construction of random DNA fragment libraries; and other molecular biology experiments.

Protocol

1. Perform on ice. Assemble the following components in an RNase-free tube:

Components	100 μL Reaction
RNA	~10 μg
10 x DNase I Buffer	10 μL
DNase I (RNase-free)	1 μL
Nuclease-free H ₂ O	To 100 μL

2. Mix gently, spin briefly, and incubate at **37 °C for 10 min**.

Shipping and Storage

Storage: **-30 ~ -15 °C**, Shipping: **≤ 0 °C**

Precautions

1. When removing DNA from RNA samples, add **Murine RNase Inhibitor** to protect RNA from degradation.
2. The optimal dosage of DNase I may need optimization under specific experimental conditions.
3. Inactivate DNase I by adding **5 mM EDTA** and heating at **65 °C for 10 min**, or by phenol/chloroform extraction. Activity is strongly inhibited by: metal ion chelators, millimolar Zn^{2+} , 0.1% SDS, reducing agents (DTT, β -mercaptoethanol), and salt concentrations above 50–100 mM.