DNase I, RNase-Free, HC

Instruction for Use

Cat. No./Spec.: E1018-A/1,000 U

Concentration: 50 U/μL

Product Description

DNase I (without RNase) is an endonuclease that can cleave both single-stranded and double-stranded DNA. It hydrolyzes phosphodiester bonds, producing single deoxyribonucleotides and oligodeoxyribonucleotides with 5'-phosphate groups and 3'-OH groups.

The activity of this enzyme is strictly dependent on Ca^{2+} and is activated by Mg^{2+} or Mn^{2+} ions. In the presence of Mg^{2+}, DNase I cuts each strand of dsDNA in a statistically random and independent manner. When Mn^{2+} is present, the enzyme almost cuts both DNA strands at the same site, resulting in DNA fragments with blunt ends or overhangs of one or a few nucleotides.

Components

Component	*E1018-A*
DNase I, RNase-Free, HC (50 U/μL)	*20 μL*
10X DNase I Buffer (Mg^{2+} Plus)	*1 mL*
EDTA (50 mM)	*1 mL*

Storage Condition & Shelf Life

Store at -20°C.

Source

Recombinant E. coli strain containing the cloned gene encoding bovine DNase I.

Unit Definition

A unit is defined as the amount of enzyme required to completely degrade 1 μg of plasmid DNA within 1 minute at 37° C.

Enzyme activity is determined in the following mixture: 10 mM Tris-HCl (pH 7.5 at 25° C), 2.5 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 1 μg of pUC19 DNA.

One unit of DNase I is equivalent to 0.3 Kunitz units.

Features

- Recombinant enzyme
- Purified from a non-animal host, with low levels of endogenous RNase

Scope of Application

- For the preparation of DNA-free RNA.

- To remove template DNA after in vitro transcription.

- To prepare DNA-free RNA before RT-PCR and RT-qPCR.

- In conjunction with DNA Polymerase I for DNA labeling through nick translation.

- For the study of DNA-protein interactions using DNase I (RNase-free) footprinting.

- To generate a library of randomly sheared DNA insert fragments. Reaction buffer containing Mn^{2+} is used.

Inhibition and Inactivation

- Inhibitors: metal chelating agents, millimolar concentrations of transition metals (such as zinc), SDS (even at concentrations below 0.1%), reducing agents (DTT and β-mercaptoethanol), and ionic strength higher than 50-100 mM.

- Inactivation is achieved in the presence of EGTA or EDTA by heating at 65°C for 10 minutes (using at least 1 mol of EGTA/EDTA per 1 mol of Mn^{2+}/Mg^{2+}).

Protocol

1. Removal of genomic DNA from RNA samples

① Prepare the reaction system in an RNase-Free tube:

Component	*Amount*
RNA	*1 μg*

10X DNase I Buffer (Mg^{2+} Plus)	*1 μL*
DNase I, RNase-Free, HC (50 U/μL)	*1 μL*
DEPC-treated Water (#R2041)	*To 10 μL*

② Incubate at 37°C for 30 minutes.

③ Add 1 μL of 50 mM EDTA and incubate at 65°C for 10 minutes. In the absence of chelating agents, RNA is hydrolyzed with divalent cations during heating. Alternatively, use phenol/chloroform extraction.

④ Use the prepared RNA as a template for reverse transcriptase.

Note:

- Use no more than 1 U of DNase I, RNase-Free, per 1 μg of RNA.

- DNase I, RNase-Free, HC can be diluted in 1X reaction buffer or in storage buffer before use to extend storage time.

- The volume of the reaction mixture and the 50 mM EDTA solution can be scaled up to obtain larger quantities of RNA. The recommended final concentration of RNA is 0.1 μg/μL.

- The RNase inhibitor RNasin (#R2011) can also be included in the reaction mixture to prevent RNA degradation.

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 the 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.

3. DNA labeling by nick-translation

① Prepare the reaction system:

Component	*Amount*
10X reaction buffer for DNA Polymerase I	*2.5 μL*
Mixture of 3 dNTPs, 1 mM each (without the labeled dNTP)*	*1.25 μL*
[α-^{32}P]-dNTP,	*1.85-3.7 MBq*
~110 TBq/mmol (3000 Ci/mmol)	*(50-100 μCi)*
*DNase I, RNase-free freshly diluted to 0.002 U/μL***	*1 μL*
DNA Polymerase I (#E1021)	*5-15 U*
Template DNA	*0.25 μg*
Water, nuclease-free (#P9021)	*To 25 μL*

Note:

** To prepare a mixture of three unlabeled dNTPs (each at 1 mM), mix 1 μL of each dNTP sample (100 mM) with 97 μL of nuclease-free water. Store at -20°C.*

*** DNase I, RNase-Free can be diluted with the 1X reaction buffer of DNA Polymerase I: 50 mM Tris-HCl (pH 7.5 at 25°C), 10 mM $MgCl_2$, and 1 mM DTT.*

② Incubate immediately at 15°C for 15-60 minutes.

③ Terminate the reaction by adding 1 μL of 0.5M EDTA, pH 8.0.

④ Take an aliquot (1 μL) to measure the efficiency of label incorporation. The expected specific activity of DNA should be at least 108 cpm/μg.

This product is for research use only.