DNase I，RNase-free

REF: EG22204S

Storage Condition

-20℃

Components

Component	Amount
DNase I, RNase-free(1 U/μl)	1000 U
10×DNase I Buffer	1.25 ml

Description

DNase I, RNase-free is an endodeoxyribonuclease that catalyzes to the same extent the degradation of both single- and double-stranded DNA randomly, and produces 5'-P terminal oligonucleotides.

The activity of DNase I is dependent on Ca^{2+}, and can be activated by bivalent metal ions Mg^{2+}, Mn^{2+}, etc. In the presence of Mg^{2+}, the enzyme can randomly recognize and cut any site on any strand of double-stranded DNA. In the presence of Mn^{2+}, nearly identical sites on the two strands of DNA can be recognized and cut, resulting in flat ends or sticky end DNA fragments with 1 to 2 nucleotides overhang.

Definition of Activity Unit

One unit is defined as the amount of enzyme which will completely degrade 1 μg of pUC19 DNA in 10 minutes at 37℃ in DNase I Reaction Buffer.

Applications

1. Removal of contaminating genomic DNA from RNA samples.
2. Degradation of DNA template in transcription reactions.
3. DNase I footprinting.
4. Nick Translation.
5. DNA library construction.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

RNase Activity

A 10 μl reaction containing 500 ng of total RNA and 1 U of DNase I, RNase-free incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Protocol

1. Digestion of genomic DNA in a sample for RT-PCR

Set up the following reaction on ice:

Reagent	Amount
RNA	1 μg
10×DNase I Buffer	1 μl
DNase I, RNase-free(1 U/μl)	1 μl
Nuclease-Free Water	up to 10 μl

Incubate for 15 min at 37℃ .

Perform one of the following procedures to inactivate DNase I:

① Add EDTA with a final concentration of 5 mM, and incubate at 75℃ for 10 min. EDTA can prevent the hydrolysis of RNA with bivalent cations in the reaction system during heating. If this step of thermal inactivation is performed, the final concentration of 2.5 mM Mg^{2+} needs to be supplemented in the downstream RT-PCR or RT-qPCR reaction system, so as to avoid excessive EDTA in the reaction system affecting the downstream RT-PCR or RT-qPCR reaction.

② Column based purification or Phenol/chloroform treatment.

2. Degradation of DNA template in transcription reactions

Perform the following procedures:

① 1 U DNase I was added to the transcription reaction system of 0.5 μg template DNA. The amount of enzyme can be optimized according to actual needs.

② Incubate for 15 min at 37℃ .

③ Column based purification or Phenol/chloroform treatment.

Notice

1. When performing RNA-related experiments, be careful to maintain an RNase-free environment.
2. When using this product to remove DNA from RNA samples, RNase Inhibitor, Murine (REF: EG20002S) can be added to the reaction system to protect RNA from degradation.
3. Avoid violent shocks when preparing samples.
4. For your safety and health, please wear a lab coat, disposable gloves, and a mask while conducting the experiment.

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