Tinzyme Co., Limited

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DNase I, Recombinant

Product Number: DI07

Shipping and Storage

2-8℃ storage, -20℃±5℃ long-term storage; After re dissolving, the sample should be stored at -20℃±5℃ to avoid repeated freeze-thaw cycles.

Component

Component	100U	500U
DNase I, Recombinant	100U	500U
25mM EDTA	500μl	2.5ml

Description

DNase I (deoxyribonuclease I) is a type of endonuclease that can randomly break down single or double stranded DNA to the same extent, generating deoxyribonucleic acid with 5'-P terminal oligonucleotides. DNase I hydrolyzes single or double stranded DNA, and its activity is highly dependent on Ca^{2+} levels and can be activated by Mg^{2+}or Mn^{2+}. Under the presence of Mg^{2+}, DNase I, Recombinant can randomly cleave any site of double stranded DNA; Under the presence of Mn^{2+}, DNase I can cleave two strands of DNA at approximately the same site, resulting in a sticky end with either a flat end or 1-2 nucleotide protrusions.

This product does not contain components such as glycerol that affect the freeze-drying process. It is a freeze-dried powder made by pre mixing DNase I and excipients. The freeze-dried powder has a uniform appearance, a block like or sponge like structure, and dense pores. Simply add RNase Free Water to quickly dissolve and use.

Features

1. Adding RNase Free Water allows for rapid re dissolution;
2. No need for cold chain transportation, can be stored at room temperature and 2-8℃;
3. The reaction can be completed without adding a reaction buffer.

Application

1. Prepare RNA samples without DNA;
2. Remove possible DNA contamination such as genomic DNA from RNA samples before RT-PCR reaction;
3. Removal of DNA templates after in vitro transcription catalyzed by RNA polymerase such as T7, T3, SP6, etc;
4. Used for Footprinting analysis of DNA protein interactions;
5. Used together with DNA polymerase I for incision translation;
6. Under the presence of divalent manganese ions, DNA fragments are fragmented to generate a random DNA fragment library;
7. Partial splicing of genomic DNA was used as a positive control in TUNEL detection of cell apoptosis.

Unit definition

The amount of enzyme required to completely degrade 1μg of pBR322 plasmid DNA within 10 minutes at 37 ℃ is defined as one active unit.

Quality control

After multiple column purification, only a clear and single target band was visible in SDS-PAGE gel detection. qPCR method detected no residual Escherichia coli DNA and no contamination of nucleic acid endonucleases and exonucleases.

Protocol

1. **Re dissolution of freeze-dried powder:** The sample is added to 100μl of RNase Free Water to prepare a 1U/μl DNase I solution. RNase Free Water can be added according to actual usage needs to prepare DNase I solutions with different active units. The remaining reagents after dissolution can be stored at -20℃±5℃.

2. Application 1: Removing contaminated genomic DNA from RNA samples

2.1. Place the sample on an ice box and prepare the following reaction system on ice:

Component	volume
Template RNA	1μg
DNase I(After re dissolution, 1U/μl)	1μl (1U)
RNase Inhibitor (40U/μl)	0.5-1μl (No need to add)
RNase Free Water	To 10μl

2.2. After mixing, centrifuge immediately and incubate at 37 ℃ for 30 minutes;

2.3. Add EDTA with a final concentration of 2.5mM and heat at 65 ℃ for 10 minutes to terminate the reaction.

Note:RNA is easily degraded when heated and can be extracted with phenol/chloroform to remove DNase, followed by ethanol precipitation of RNA.

3. Application 2: Removing DNA templates after in vitro transcription

3.1. According to each μg of DNA template, add 2U of DNase I (1U/μl after re dissolution), and note that the amount of enzyme can be optimized according to actual needs;

3.2. Incubate at 37℃ for 15 minutes;

3.3. Add EDTA with a final concentration of 2.5mM and heat at 65℃ for 10 minutes to terminate the reaction.

Note:RNA is easily degraded when heated and can be extracted with phenol/chloroform to remove DNase, followed by ethanol precipitation of RNA.

Note

1. This reagent is a freeze-dried product. If it needs to be stored for a long time after re dissolving, it is recommended to store it at -20℃±5℃ after packaging.
2. When using, place the re dissolved enzyme on ice and store it at -20℃±5℃ after use.
3. Metal ion chelating agents, such as 0.1% SDS, DTT, and mercaptoethanol, have inhibitory effects on enzymes.