Recombinant DNase I(RNase Free), Lyophilized powder

Cat.No.:HYC127

Product Description

Deoxyribonuclease I (DNase I) is an endodeoxyribonuclease that digests single-stranded or double-stranded DNA. It recognizes and cleaves phosphodiester bonds, generating monodeoxynucleotides or single- or double-stranded oligodeoxynucleotides with a phosphate group at the 5' end and a hydroxyl group at the 3' end. DNase I activity is dependent on Ca^{2+} and can be activated by divalent metal ions such as Mn^{2+} and Zn^{2+}. 5mM Ca^{2+} protects the enzyme from hydrolysis. In the presence of Mg^{2+}, the enzyme can randomly recognize and cleave any site on either DNA strand. In the presence of Mn^{2+}, however, it can recognize both DNA strands simultaneously and cleave at nearly identical sites, forming blunt-ended or sticky-ended DNA fragments with one to two nucleotide overhangs.

Components

Components	2.5K U
Recombinant DNase I(RNase Free), Lyophilized powder	2.5KU
10×DNase I Buffer	2.5 mL

Storage

Store at-15°C ~-25°C for 2 years.

Unit Definition

One unit (U) of activity refers to the amount of enzyme required to completely degrade 1 μg of pBR322 DNA at 37°C in 10 minutes.

Source

Bovine pancreatic DNase I was expressed in a yeast expression system and purified.

Quality Control

RNase activity: 5 U of this product and 1.6 μg of MS2 RNA were reacted at 37°C for 4 hours. The RNA electrophoretic bands did not change.

Instructions

1. To prepare DNase I stock solution: Dissolve DNase I dry powder (2500 U) in 500 µL of RNase-free ddH_2O, mix gently, and aliquot. Store at -25°C to -15°C (stable for 12 months), removing aliquots as needed for experiments. Note that DNase I stock solution stored at -25°C to -15°C should not be frozen and thawed more than five times. Alternatively, store DNase I stock solution at 2°C to 8°C (stable for 2 months).
2. Prepare the reaction solution in an RNase-free reaction tube according to the following ratios:

Components	Volume
10×DNase I Buffer	1µL
DNase I stock solution	1 U per µg RNA*
RNA	X µg
RNase-free ddH_2O	Up to 10µL

*Calculate the volume of DNase I stock solution required based on the amount of RNA.

3. Incubate at 37°C for 15 minutes.
4. Add 0.5M EDTA to a final concentration of 2.5mM-5mM, then heat at 65°C for 10 minutes to terminate the reaction. The sample can then proceed directly to the next step, such as reverse transcription.

Notes

1. This product is RNase-free DNase I, in dry powder form. To avoid loss during the dissolution process, carefully open the bottle cap and slowly add RNase-free ddH_2O directly into the bottle. Alternatively, leave the bottle cap open and inject RNase-free ddH_2O directly into the bottle using an RNase-free syringe. Invert to mix thoroughly to dissolve the DNase I, then aspirate the solution using an RNase-free syringe (RNase-free syringes are required).
2. When preparing the DNase I stock solution, do not vortex; gently invert to mix.
3. 0.5M EDTA is required.
4. Use 1 unit of DNase I stock solution per µg of RNA. If the RNA amount is less than 1 µg, use 1 unit of DNase I stock solution.
5. Keep the enzyme on ice when using to avoid repeated freezing and thawing.