DNase I (RNase Free)

User's Instruction

Description

DNase I (RNase Free) is an endonuclease that decomposes single- or double-stranded DNA to produce 5'-P-terminal oligonucleotides. As the protease is almost completely removed, the stability of DNase I in pH neutral region is improved. In addition, RNase inhibitor is added to DNase I to inhibit the residual RNase in RNA samples, so it can effectively inhibit RNase degradation of RNA during the extraction. The DNase I activity can be inactivated by one-step heating after the reaction is terminated by DN Stop Buffer.

Kit Contents

	1000U
1. DNase I (RNase Free) (10 U/μl)	100 μl
2. 10 × DN Buffer	500 μl
3. 10 × DN Stop Buffer	500 μl

- DNase I (RNase Free) (10 U/μl) contains 20 U/μl RNasin. Therefore, it is not necessary to add additional RNasin.

Unit Definition

Using calf thymus DNA as substrate, under the condition of 25°C and pH 5.0, the enzyme amount needed to increase the 260 nm absorbance of the reaction solution by 0.001 in 1 minute is defined as one active unit (Kunitz unit).

Protocol

1. Set up the reaction as the following table:

Component	Volume
RNA	10-50 μg
10 × DN Buffer	5 μl
DNase I (RNase Free) (10 U/μl)	1 μl

ddH_2O (RNase Free)	Up to 50 µl

Note: If the RNA sample contains more than 5 µg genomic DNA contamination, please use 2 µl of DNase I (RNase Free) (10 U/µl).

2. After incubation at 37°C for 10 min, the genomic DNA is digested and removed.
3. After incubation, add 5 µl 10 × DN Stop Buffer, mix evenly, place at room temperature for 1 min, and heat at 75°C for 10 min to inactivate DNase I. The sample can be directly used for the downstream reverse transcription.

Inactivation

Generally, DNase I can be inactivated by heating. If it is necessary to remove the residual denatured protein, the RNA sample can be extracted and precipitated with phenol chloroform after digestion at 37°C (without heating).

Note

- 10 × DN Stop Buffer contains chelating agent to remove divalent cations. Before heat inactivation, 5 µl 10 × DN Stop Buffer must be added and mixed evenly, and then heat inactivation is carried out. Otherwise, it will lead to RNA degradation.
- When the final RNA sample is processed for one-step reverse transcription reaction, the addition amount should be less than 20% (for example, the addition amount in 20 µl reverse transcription system should be less than 4 µl)

Storage

Minimum shelf life is 2 years at -20°C.

Only for research and not intended for treatment of humans or animals