dsDNase

REF: EG20206S

Storage Condition

-20℃

Components

Component	Amount
dsDNase(1 rxn/μl)	50 μl
10×dsDNase Buffer	200 μl

Description

dsDNase is a deoxyribonuclease that hydrolyzes phosphodiester bonds in dsDNA, generating oligonucleotides with 5'-phosphate and 3'-hydroxyl ends. dsDNase specifically hydrolyzes dsDNA or the DNA strand in DNA-RNA hybrid chains, while having no effect on RNA and single-stranded DNA. dsDNase is heat-labile and can be rapidly inactivated at 55 ℃ with DTT. It is primarily used to eliminate genomic DNA contamination in RNA samples before reverse transcription. Compared to the traditional method of using DNase I to eliminate genomic DNA contamination, dsDNase does not require additional EDTA, reduces RNA damage, saves experimental time, and ensures accurate quantification of RNA. The activity of dsDNase can be inhibited by EDTA, SDS, DTT, β-mercaptoethanol and high salt concentrations.

Definition of Activity Unit

One unit increases the absorbance at 260 nm by 0.001 OD per min at 25℃ and pH 5.0 with excess large molecular weight DNA as the substrate according to the assay method of Kunitz.

Quality Control Assays

Protein Purity

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

RNA Activity

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

Function Testing

After using 1 μl of dsDNase to remove genomic DNA contamination from RNA sample, proceed with RT-qPCR amplification. The removal efficiency of genomic DNA is ≥99.9%, and the RNA levels are unaffected by dsDNase treatment.

Protocol

1. Prepare the following reaction mixture on ice:

Reagent	Amount
dsDNase	1 μl
10×dsDNase Buffer	1 μl
Template RNA	X μl
Total RNA	1 pg~5 μg/10 μl
or mRNA	0.1 pg~500 ng/10 μl
or Specific RNA	0.01 ng~500 ng/10 μl
Nuclease-Free Water	To 10 μl

2. Mix gently and spin down, then incubate at 37℃ for 2~5 minutes.

3. Add a final concentration of 10 mM DTT, and incubate at 65℃ for 2 minutes to inactivate dsDNase. Then quickly place the RNA on ice for subsequent experiments.

Note: for long-term RNA storage, please keep it at -80℃ and avoid repeated freeze-thaw cycles.

Notice

1. If the downstream application of RNA samples is reverse transcription, the inactivation step can be performed without DTT which is already included in most reverse transcription reagents.

2. To prevent RNA degradation, add an appropriate amount of RNase inhibitor to the reaction mixture.

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com