Thermolabile dsDNase

T751064

Storage: -20℃. Avoid freeze/thaw cycles.
Shipping: Shipped with ice packs.

Introduction:

Thermolabile dsDNase is a nuclease endonuclease that cleaves phosphodiester bonds in DNA, generating oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. dsDNase specifically digests double-stranded DNA (dsDNA) without digesting single-stranded DNA, primers, probes, or RNA. dsDNase is thermolabile and can be rapidly inactivated at 55°C. dsDNase is mainly used for rapid removal of genomic DNA contamination from RNA samples prior to reverse transcription experiments. Compared with the traditional method of removing genomic DNA contamination using DNase I, it eliminates the need for additional EDTA inactivation, reduces RNA damage, saves experimental time, and ensures the accuracy of RNA quantification.

Components and Specifications:

T751064	Components	Appearance	50T	5*50T	Storage
T751064A	Thermolabile dsDNase	Liquid	50 μl	5×50 μl	-20 ℃. Avoid freeze/thaw cycles.
T751064B	10× dsDNase Buffer	Liquid	200 μl	5×200 μl	-20 ℃. Avoid freeze/thaw cycles.

Activity Definition:

One unit (U) of activity is defined as the amount of enzyme that causes an increase in absorbance of 0.001 per minute at 260 nm, using excess high-molecular-weight DNA as substrate, at 25 °C and pH 5.0, according to the Kunitz method.

Inhibition and Inactivation:

Inhibition conditions: Metal ions, EDTA, SDS, DTT, β-mercaptoethanol, high salt concentration, etc. will inhibit the activity of dsDNase.
Inactivation conditions: Incubate at 55°C for 5 min.

RNase Activity Assay:

The enzyme was incubated with RNA substrate at 37°C for 1 hour, and no degradation of the RNA substrate was detected by gel electrophoresis.

Functional Assay:

The product was tested for removal of genomic DNA contamination from RNA samples followed

by RT-qPCR amplification. The efficiency of genomic DNA removal is ≥99.9%, and RNA quantity is not affected by dsDNase treatment.

Usage:

1. Prepare the following reaction mixture on ice:

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

2. Mix the reaction mixture gently by pipetting, then incubate the mixture at 37°C for 2–5 min.
3. Heat-inactivate at 65°C for 2 min, then immediately place the obtained RNA on ice for subsequent experiments. For long-term storage, keep at -80°C and avoid repeated freeze-thaw cycles.

Notes:

1. If the RNA sample is used for downstream RT-PCR with a target gene length ≥3 kb, add DTT to a final concentration of 10 mM before the inactivation step.
2. To avoid RNA degradation, an appropriate amount of RNase Inhibitor can be added to the reaction system.

Troubleshooting:

Problem Description: Amplification observed in NTC control of RT-qPCR experiment.

Cause:

1. Severe gDNA contamination with insufficient reaction time;
2. High concentration of inhibitors in RNA template affecting dsDNase activity.

Solution:

1. Extend incubation time to 5 min;
2. Wash the RNA template with 75% anhydrous ethanol and dissolve in nuclease-free water.