dsDNase

Catalog Number EN0771

Pub. No. MAN0012879 **Rev.** B00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Sheets (SDSs) are available from **thermofisher.com/support.**

Contents and storage

Contents	Amount	Storage
dsDNase	50 µL	-25 °C to -15 °C
10X dsDNase Buffer	100 µL	
Water, nuclease-free	1.25 mL	

Description

Thermo Scientific dsDNase is an engineered shrimp DNase designed for rapid and safe removal of contaminating genomic DNA from RNA samples. It is an endonuclease that cleaves phosphodiester bonds in DNA to yield oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. Highly specific activity towards double-stranded DNA ensures that RNA and single-stranded DNA such as cDNA and primers are not cleaved. dsDNase is easily inactivated by moderate heat treatment (55 °C). These features make dsDNase an excellent choice for gDNA elimination prior reverse transcription. It allows for dramatically simplified workflow which combines genomic DNA elimination and cDNA synthesis into one-tube procedure.

Applications

Genomic DNA removal from RNA samples prior first strand cDNA synthesis, RT-PCR and RT-qPCR.

General Recommendations

- The recommended reaction volume is 10 µL. It should not exceed the input limit of the following RT reaction.
- Use RNase-free tubes, gloves and filter tips.

Source

Pichia pastoris strain expressing a gene for a recombinant DNase.

Unit definition

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

Storage Buffer

The enzyme is supplied in 20 mM Tris-HCl (pH 7.5 at 25 °C), 2.0 mM $MgCl_2$, 10 mM NaCl, 0.01 % (v/v) Tween 20, 50 % (v/v) glycerol.

Inhibition and Inactivation

Inhibitors: metal chelators, transition metals (e.g., Zn) in millimolar concentrations, SDS (even at concentrations less than 0.1 %), reducing agents (DTT and β-mercaptoethanol), high salt concentration.

Protocol

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a master mix by mixing water, buffer and dsDNase. Aliquot the master mix into individual RNase-free tubes and add template RNA.

1. Mix the following components in RNase-free tubes.

Component	Amount
10X dsDNase Buffer	1 µL
dsDNase	1 µL
Template RNA	Up to 8 µL
Water, nuclease-free	To 10 µL
Total volume	10 µL

2. Gently mix the samples and spin down.
3. Incubate for 2 min at 37 °C in the preheated thermomixer or water bath.

Note. If RNA sample is to be used for RT-PCR amplification of fragments ≥ 3 kb, perform dsDNase inactivation by incubating the sample at 55 °C for 5 min in the presence of 10 mM DTT.

4. Chill on ice, briefly centrifuge and place back on ice.
5. Add reverse transcription reagents directly to the same tube and proceed with first strand cDNA synthesis protocol according manufacturer's instructions.

The product of the first strand cDNA synthesis can be used directly in PCR and qPCR or stored at -20 °C for up to one week. For longer storage, -70 °C is recommended. Avoid freeze/thaw cycles of cDNA.

Troubleshooting

Problem	Solution
RT-PCR/RT-qPCR product in RT-minus control	The appearance of a PCR product in the negative control reaction (RT-) indicates that the reaction is still contaminated with DNA. To completely remove residual DNA, prolong RNA incubation with dsDNase step up to 5 minutes. Note that trace amounts of agents used in RNA purification protocols (e.g., SDS, EDTA, guanidine salts, phosphate, pyrophosphate, polyamines, spermidine) may remain in solution and inhibit dsDNase activity. To remove trace contaminants, re-precipitate the RNA with ethanol, wash the pellet with 75% ethanol and dissolve in nuclease-free water.

Revision history: Pub. No. MAN0012879

Revision	Date	Description
B00	2024-10-30	Updated buffer composition, CoA part is removed

Limited product warranty

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