DNase I (RNase-Free)

Cat.no. RTT-BD11

Product Size:

Contents	Size
DNase I, RNase-Free (lyophilized)	1500 Kunitz units*
Buffer DRDD	4 ml
RNase-Free Water	1 ml

Storage: The RNase-Free DNase Set is shipped at room temperature and should be stored immediately upon receipt at 2–8 °C. The buffer and lyophilized enzyme can be stored for at least 9 months at 2–8 °C.

Note: Kunitz units are the most commonly used units for measuring DNase I, and 1 Kunitz unit is defined as the quantity of DNase I that causes an increase of 0.001 per minute per milliliter in A_{260} at a temperature of 25 °C and pH of 5.0 when highly polymerized DNA is used as the substrate.
(Kunitz, M. [1950] J. Gen. Physiol. 33, 349, and 363)

Introduction

RNase-free DNase I is an endonuclease that efficiently digests single-stranded and double-stranded DNA to a mixture of short oligonucleotides and mononucleotides. It hydrolyzes phosphodiester bonds to produce monodeoxyribonucleotides and oligodeoxyribonucleotides with 5'-phosphate and 3'-OH groups.

Protocol

The RNase-Free DNase Set enables the efficient on-column digestion of DNA during RNA purification by using EasyPrep RNA extraction kits. Dissolve the lyophilized DNase I (1500 U) in 550 µL of the RNase-free ddH_2O. Mix gently by inverting, and do not vortex. Divide the mixture into single-use aliquots, and store the aliquots at –20 °C for up to 9 months. Thawed aliquots can be stored at 2–8 °C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Application Example:

Digestion of Genomic DNA in a Sample for RNA-PCR

1. Prepare the following reaction mixture.

Content	Vol.
Total RNA	20–50 µg
DRDD Buffer (supplied)	5 µL
DNase I (RNase-free)	4 µL
RNase Inhibitor	0
DEPC-treated water	up to 50 µL

2. Incubate for 20–30 minutes at 37 °C.
3. Perform one of the following procedures to inactivate DNase I

   A · Heat treatment

   (1) Add 2.5 µL of 0.5 M EDTA, and incubate the mixture at 80 °C for 2 minutes.

   (2) Increase reaction volume up to 100 µL with DEPC-treated water.

   B · Phenol/Chloroform extraction

   (1) Add 50 µL of DEPC-treated water to 50 µL of the reaction mixture, and mix the resultant mixture with equal volumes (100 µL) of phenol/chloroform/isoamyl alcohol (25:24:1).

   (2) Centrifuge at 12,000 rpm for 5 minutes at room temperature and then transfer the upper layer to a new tube.

   (3) Add an equal amount of chloroform/isoamyl alcohol (24:1) and mix.

   (4) Centrifuge at 12,000 rpm for 5 minutes at room temperature and then transfer the upper layer to a new tube.
4. Add 10 µL of 3 M sodium acetate and 250 µL of chilled ethanol to the solution; then, mix the resultant mixture. Maintain the mixture for 20 minutes at −80 °C.
5. Centrifuge at 12,000 rpm for 10 minutes at 4 °C. Remove the supernatant.
6. Wash the precipitate with chilled 70% ethanol. Centrifuge at 12,000 rpm for 5 minutes at 4 °C and then remove the supernatant.
7. Dry the precipitate.
8. Dissolve the precipitate in a suitable amount of DEPC-treated water.
9. Confirm that genomic DNA is completely removed using electrophoresis and measure the RNA concentration. If the genomic DNA is not removed completely, increase the amount of enzyme or extend the reaction time.

Note: Buffer DRDD is optimized for on-column DNase digestion. The buffer is also suitable for efficient DNase digestion in solution. In some cases, the vial of DNase may appear empty because of lyophilized enzymes sticking to the septum. To avoid loss of DNase, do not open the vial. Instead, inject RNase-free water into the vial by using a needle and syringe, invert the vial to dissolve the DNase, and remove the dissolved DNase by using the syringe and needle. Insoluble material may remain when dissolving DNase. This material does not affect DNase performance

The product is for research only, not for diagnostic and clinical use.