DNase I (RNase-Free)

Molecular Biology Grade

Store at -20°C

Cat. No. YDN02
DNase I (2,000 units/ml) : 500 µl x 2
DNase I Reaction Buffer : 5 ml x 2

Cat. No. YDN10
DNase I (2,000 units/ml) : 500 µl x 10
DNase I Reaction Buffer : 5 ml x 10

Cat. No. YDN20
DNase I (2,000 units/ml) : 500 µl x 20
DNase I Reaction Buffer : 5 ml x 20

Cat. No. YDN60
DNase I (2,000 units/ml) : 500 µl x 60
DNase I Reaction Buffer : 5 ml x 60

Format: Liquid (RNase free)
Source: Bovine pancreas from USDA approved locations
Purity: ≥95% by SDS-PAGE (chromatographically purified)
Activity: ≥ 1800 units/mg protein according to Kunitz (25°C)

Storage Conditions

DNase I (RNase-Free) shall be stored at -20°C in a constant freezer and protected from frequent temperature changes.

Description

DNase I is provided in RNase-free storage buffer. DNase I is a chromatographically purified, pyrimidine-specific endoribonuclease that degrades both single-stranded and double-stranded DNA, leaving RNA intact. It's commonly used for DNA digestion in RNA related applications.

Unit definition

One unit is defined as the amount of enzyme that causes an increase in A260 of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate.

Quality Control

RNases: (not detected).

Applications

Removal of DNA from protein and RNA preparations.
Single- and double-stranded DNA digestion in RNA purification.
Removal of contaminating genomic DNA from RNA samples.
Degradation of DNA template in transcription reactions.
DNA footprinting and mapping of DNase I sensitive sites.
Nick translation of DNA.

Following protocols ensures complete DNA removal from RNA extracted using Real Biotech Corp's RNA extraction kits for use in DNA sensitive downstream applications.

In Column DNase I Digestion Protocol (Following RNA Binding):

IMPORTANT: The amount of DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform DNA Digestion In Solution to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may effect RNA integrity and reduce yield.

1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14,000-16,000 x g for 30 seconds.
2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube.
3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:
   DNase I (2U/µl) : 5 µl
   DNase I Reaction Buffer : 45 µl
   Total volume : 50 µl
4. Gently pipette the DNase I solution to mix (DO NOT vortex) then add DNase I solution (50 µl) into the CENTER of the RB column matrix.
5. Incubate the column for 15 minutes at room temperature (20-30°C) then proceed with the RNA Wash step.

DNA Digestion In Solution Protocol (Following RNA Elution):

1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:
   RNA in RNase-free water : 1 - 40 µl
   DNase I (2U/µl) : 0.5 µl/µg RNA
   DNase I Reaction Buffer : 5 µl
   RNase-free water : add to final total volume 50 µl
   Total volume : 50 µl
2. Gently pipette the DNase I reaction solution to mix (DO NOT vortex) then incubate the microcentrifuge tube at 37°C for 15-30 minutes.
3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) then incubate at 65°C for 10 minutes.
4. Repurify the RNA sample by adding 5 volumes of RB Buffer to the DNase I reaction (e.g. 250 µl of RB Buffer to 50 µl of DNase I reaction) then mix well by vortex. Add 1 volume of 70% ethanol to 1 volume of sample mixture then mix well by vortex. Transfer all of sample mixture to a new RB Column. Centrifuge at 14,000-16,000 x g for 1 minute then discard the flow through. Proceed with the RNA Wash step.

RBC
It's the Joy of Discovery

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