Applications

DNase I used for isolation of DNA-free RNA to ensure that RT-PCR templates are free of genomic DNA.

Unit Definition

One unit is defined as the amount of enzyme which will completely degrade 1μg of pBR322 DNA in 10 minutes at 37˚C.

Storage Buffer

10mM Tris-HCl, 2mM CaCl2, 50% glycerol, pH 7.6 at 25℃.

Inhibitors

EGTA, EDTA, SDS, salt concentrations > 100mM will reduce DNase activity.

Suggested Procedure

1. For digestion of genomic DNA in RNA sample, prepare the reaction mix below:

Components	Volume
RNA	X μg
10X Reaction Buffer	1μl
DNase I	1u per μg RNA*
Nuclease-free Water	up to 10μl**

* Calculate the volume of DNase I that needs to be added based on the amount of RNA.

* Use 1u DNase I per μg of RNA. However, if the RNA is less than 1 μg, please use 1u DNase I.

** 1U of RNase Inhibitor, typically can also be included in reaction mixture to prevent RNA degradation.

2. Incubate at 37℃ for 15 minutes.
3. Add 0.5M EDTA to the final concentration of 2.5mM ~ 5mM, and heat at 65℃ for 10 minutes to stop the reaction. The concentration of EDTA has to be taken into account for all subsequent applications.

Note

- DNase I is sensitive to physical denaturation. Gently mix preparations by inversion; do not vortex.
- DNase I can be diluted with 1X reaction buffer: 20mM Tris-HCl (pH 7.5 at 25˚C), 1mM $MgCl_2$ and glycerol 50%.

Quality Control Assay Data

5U of DNase I with 1.6 μg MS2 RNA for 4 hours at 37℃ yields no degradation as determined by agarose gel electrophoresis.

Signs

Signs	Definitions	Signs	Definitions
	Temperature range on product use		Name and address of the manufacturer of the product
RUO	For Research Use Only		
	Product shipping conditions	REF	Product technical code

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Deoxyribonuclease I (DNase I)

(RNase-free (500u))

REF **MO5402** **Quantity: 5u/µl**

Wet or Dry ice RUO **-20 °C**

Components

Contents	Quantity/ Volume	Store Temperature
Deoxyribonuclease I, (500u)	100µl	-20°C
10X DNase I Buffer	1ml	-20°C
EDTA (0.5M)	100µl	-20°C

Description

Deoxyribonuclease I (DNase I) is an endodeoxyribonuclease that can digest single- or double- stranded DNA. It recognizes and cleaves phosphodiester bonds to produce monodeoxynucleotides or single- or double- stranded oligodeoxynucleotides with phosphate groups at the 5'- terminal and hydroxyl at the 3'- terminal. The activity of DNase I depends on Ca^{2+} and can be activated by divalent metal ions such as Mn^{2+} and Zn^{2+}. 5mM Ca^{2+} protects the enzyme from hydrolysis. In the presence of Mg^{2+}, the enzyme could randomly recognize and cleave any site on any strand of DNA. In the presence of Mn^{2+}, the double strands of DNA can be simultaneously recognized and cleaved at almost the same site to form flat end DNA fragments or sticky end DNA fragments with 1-2 nucleotides protruding. Bovine Pancreas DNase I was expressed in yeast expression system and purified.