

DNase I (RNase-free)

Cat. No. GD201

Storage: at -20°C for two years

Concentration: 3 units/ μ l

Description

Deoxyribonuclease I (DNase I) is an endonuclease that digests single- and double-stranded DNA and chromatin (reaction rate is restricted by DNA association with histones). It functions by hydrolyzing phosphodiester bonds, producing mono and oligodeoxyribonucleotides with a 5'-phosphate and a 3'-hydroxyl group. Its activity depends on Ca^{2+} and it is activated by Mg^{2+} or Mn^{2+} . DNase I with Mg^{2+} cleaves randomly double-stranded DNA at any site. DNase I with Mn^{2+} cleaves two DNA strands at approximately the same site to form sticky ends with 1-2 nucleotide overhangs or form blunt ends.

Molecular Weight

32 kDa (monomer)

Unit Definition

One unit is the amount of enzyme required to completely degrade 1 μ g pBR322 plasmid DNA in 10 minutes at 37°C.

Activity Test Condition

40 mM Tris-HCl (pH8.0), 10 mM MgSO_4 , 1 mM CaCl_2 , 1 μ g of pBR322 DNA.

Purity

Free of other DNA endonucleases and exonucleases, free of RNase.

Storage Buffer

50 mM Tris-acetate (pH7.5), 10 mM CaCl_2 , 50% (v/v) glycerol.

10×Reaction Buffer

100 mM Tris-HCl (pH7.5 at 25°C), 100 mM MgCl_2 , 1 mM CaCl_2 .

Components

DNase I (3 units/ μ l)	1500 units
10×DNase I Reaction Buffer	2×1 ml
200 mM EDTA	1 ml

Procedures

1. Add the following components to an RNase-free microcentrifuge tube:

Component	Volume
RNA	as required
10×DNase I Reaction Buffer	1 μ l
DNase I	1 unit/ μ g RNA
RNase-free Water	Up to 10 μ l

2. Incubate at 37°C for 30 minutes.
3. Terminate reaction by the addition of 0.5 μ l of 200 mM EDTA solution.
4. Incubate at 65°C for 10 minutes to inactivate DNase I.
5. Treated RNA sample is ready for reverse transcription.

Applications

- Preparation of DNA-free RNA samples.
- Removal of genomic DNA or other possible DNA contamination in RNA samples before RT-PCR.
- Removal of DNA templates after in vitro RNA transcription catalyzed by RNA Polymerases such as T7, T3, SP6, etc.
- Study of DNA-protein Interactions by DNase I footprinting.
- Nick translation.
- Generation of a library containing random fragments of DNA.
- Generation of partially cleaved genomic DNA as a positive control in cell apoptotic TUNEL assay.

Note

- Use 1 unit DNase I for per μ g RNA. If the amount of RNA is less than 1 μ g, use 1 unit DNase I.
- At least 1mM EDTA is needed for 1 mM Mg^{2+} to terminate reaction. In the reaction system, the working concentration of Mg^{2+} is 10 mM, so 0.5 μ l of 200 mM EDTA solution is required to terminate the reaction. The final concentration of EDTA is 10 mM.

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