DNase I (RNase-free)

Cat. No. GD201
Storage: at -18°C or below for two years
Concentration: 3 units/μl

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 μg 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 μg 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/μl)	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 μl
DNase I	1 unit/μg RNA
RNase-free Water	Up to 10 μl

2. Incubate at 37°C for 30 minutes.
3. Terminate reaction by the addition of 0.5 μl 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 during RNA sample preparation.
- 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 μg RNA. If the amount of RNA is less than 1 μg, 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 μl of 200 mM EDTA solution is required to terminate the reaction. The final concentration of EDTA is 10 mM.

Service telephone +86-10-57815020
Service email complaints@transgen.com