DNase I

GE012.0100 – 100 mg

DNase I (Deoxyribonuclease I, 29kDa monomer) is an endonuclease that cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide, to release di-, tri-, and oligonucleotide products (on average producing tetranucleotides) with 5'-phosphorylated and 3'-hydroxylated ends. DNase I acts on single-stranded DNA, double-stranded DNA, RNA-DNA hybrids, and chromatin.

Quantity

100 mg lyophilized powder purified from purified from *Escherichia coli* harbouring an MBP fusion clone of Bovine Pancreatic DNase I.

Storage

Store lyophilized powder at -20ºC for up to 1 year.

For convenience, we recommend to reconstitute DNase I in 1,0 ml of a storage buffer (not included) and prepare aliquots (e.g. in RNase-free 10 mM Tris-HCl (pH 7.5), 10 mM CaCl2 10 mM MgCl2, 50% (v/v) glycerol) at a final concentration of 100mg/ml. Note that DNase I is sensitive to physical denaturation, therefore, mix gently by inversion and do not vortex. Aliquot and store at -20ºC.

Applications

Removal of residual genomic DNA from RNA samples, degradation of DNA template in transcription reactions, DNase I footprinting, Nick Translation.

Specifications

Free of RNases. Specific Activity >2000 Kunitz units per mg.

Quality Control

Functionally tested for digestion of template DNA after *in vitro* transcription.
Specific activity has been assayed by degradation of 1µg of pUC19 in 40mM Tris-HCl (pH 8.0); 10mM $MgSO_4$, 1mM $CaCl_2$. Absence of ribonuclease activity was confirmed by appropriate assays.

Enzyme activity

For maximal activity, DNase I requires bivalent cations Ca^{2+} and Mg^{2+}. pH optimum is in the range of 7.5 to 8.0. It is therefore recommended to digest DNA using a reaction buffer (not included) containing both cations (e.g. 10x reaction buffer = RNase-free 100 mM Tris-HCl (pH 7.5 at 25°C), 25 mM MgCl2, 1 mM $CaCl_2$).

Inhibition

DNase I is inhibited by metal chelators, transition metals (for example Zn) in mM concentrations, SDS (at concentrations even below 0,1%), reducing agents (DDT, β-mercaptoethanol), and ionic strength above 50-100mM.

Inactivation

DNase I can be heat-inactivated at temperatures above 65ºC.

Suggested Protocol

1) Add the sample to be treated in 1x reaction buffer, using RNase-free ddH_2O to make up final volume of 100 µl.
2) Add 1 µl of DNase I solution (100mg/ml) and incubate at 37ºC for 10 minutes.
3) Add 1 µl of 0,5M EDTA and heat-inactivate at 75ºC for 10 minutes.

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