KACTUS

1 Broadway
Cambridge, MA 02142
United States

Tel: (617) 665-7333
support@kactusbio.us
kactusbio.com

DNase I, GMP-Grade

Catalog #GMP- DNI-EE001

Storage Condition -20°C ± 5°C for 24 months. Avoid repeated freeze/thaw cycles.
Form Liquid
Source An *E. coli* strain that carries DNase I gene from *Bovine Pancreatic*
Storage Buffer 10 mM Tris-HCl (pH7.6), 2 mM $CaCl_2$, 50% Glycerol
Concentration 4U/μL
Unit Definition One unit is the amount of enzyme required to completely degrade 1 μg of pBR322 DNA in 10 minutes at 37 °C in DNase I reaction buffer.

Product Contents

- DNase I (RNase-free, 4U/μL)

Product Description

DNase I is an endonuclease that digests single- and double-stranded DNA to mono- and oligonucleotides containing 5' phosphate and 3'-hydroxylated ends. It is an essential enzyme for the efficient digestion of DNA during RNA purification, suitable for all molecular diagnostics and *in vitro* mRNA synthesis applications.

Applications

- *In vitro* transcription (IVT)
- Removal of DNA from RNA samples
- DNase I footprinting

Quality Control Statement

KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. It is suitable for mRNA therapeutics and manufacturing of mRNA vaccines. Regulatory support documents are available. Please contact support@kactusbio.us for more information.

Quality Control Release Criteria

Assay	Criteria
Activity (Plasmids Degradation)	≥ 4kU/mL
Purity (SEC-HPLC)	≥ 95%
Endotoxin	≤ 10EU/mL
Residual RNase	Negative
Residual Protease	Negative
Residual Host Cell Protein	≤ 20ng/mg
Residual Host Cell DNA	≤ 100pg/mL
Residual Heavy Metal	≤ 10 ppm
Bioburden	≤ 1CFU/10mL

Protocol for Degradation of DNA Template after *In Vitro* Transcription Reaction

1. After transcription, add 2U of DNase I to a 20μL transcriptional reaction per 1μg DNA template.
2. Incubate at 37°C for 15 minutes.
3. Add EDTA to a final concentration of 5 mM as RNA hydrolyzes during heating with divalent cations in the absence of a chelating reagent.
4. Incubate at 65°C for 10 minutes for heat inactivation of DNase I.
   Note: Phenol/chloroform extraction followed by ethanol precipitation of RNA can also be used to remove DNase I.

Protocol for *In Vitro* Transcription

1. Prepare the following reaction mixture

Reagent	Quantity
5X Transcription Buffer-1 (included with T7 RNA Polymerase)	4μL
CTP/GTP/ATP/UTP (100mM each)	2μL each
Murine RNase Inhibitor (40U/μL)	1μL
Pyrophosphatase, Inorganic (0.1U/μL)	1μL
T7 RNA Polymerase (50U/μL)	2μL
Template DNA	1μg
RNase-free Water	Up to 20μL

2. Incubate at 37°C for 1-2 hours.
3. After transcription, add 2U DNase I to digest DNA template for 15 minutes at 37°C.
4. Inactivate DNase I by phenol/chloroform extraction.

Notes

- Divalent metal ions such as Mg^{2+} and Ca^{2+} are necessary for DNase I activity, while DNase I is inhibited by monovalent metal ions such as Na^+ and K^+.