KACTUS

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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.

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

This product has been filed with the FDA Drug Master Files and is assigned DMF #038032. KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. The production is antibiotic- and animal-free.

Quality Control Release Criteria

Assay	Criteria
Activity (Plasmids Degradation)	4.0~8.0 kU/mL
Purity (SEC-HPLC)	≥ 95%
Endotoxin	≤ 1.2EU/mL
Residual RNase	Negative
Residual Protease	Negative
Residual Heavy Metal	≤ 10 ppm
Residual Nickel Salt	≤ 10 ppm
Bioburden	≤ 1CFU/10mL
Concentration	0.190 mg/ml±20%

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 (120U/μL)	0.5μL
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^{+}.