DNase I (Pharmaceutical Grade)

DN102

Storage

Store at -15 ~ 25 ℃. Avoid exposure to frequent temperature changes.
See the expiration date on the Product Information Label.

Contents

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- DNase I (Pharmaceutical Grade)

Shipping Condition

Frozen on dry ice.

Introduction

DNase I is an endonuclease derived from bovine pancreas that will degrade double-stranded DNA in the presence of divalent cations, producing 3″-OH oligonucleotides. Mg^{2+} in the reaction solution causes the enzyme to produce nicks in double-stranded DNA, while in the presence of Mn^{2+}, DNase I cleaves both strands of the DNA. DNase I is useful in nick translation for introducing single-stranded nicks that serve as primer sites for initiation of DNA synthesis and for cloning random DNA fragments by cleaving double-stranded DNA. DNase I is a chromatographically pure preparation. It is offered as lyophilized powder. For greatest stability, it is important that DNAse be dissolved at a concentration of at least 1mg/ml in 50% Glycerol with 20mM Tris-HCl, pH 7.5 and 1mM $MgCl_2$. This solution can be stored at -20°C for at least a year.

Recommended 1X Reaction Buffer

40mM Tris-HCl (pH 8.0), 10mM $MgSO_3$, 10mM $CaCl_2$.

Heat Inactivation

10 minutes at 65°C in the presence of Stop Solution.

Inhibitors

EGTA; EDTA; Salt concentration >100mM will reduce DNase activity.

Requirement

CA^{2+} and Mg^{2+} or Mn^{2+}

Source

Bovine pancreas.

Stop Solution

20mM EDTA (pH 8.0)

Unit Definition

One Kunitz unit causes as increase in absorbance at 260nm of 0.001 per minute per ml at 25°C pH 5.0 when acting on highly polymerized DNA in the presence of ionized magnesium and calcium.

Procedure

1. Add to an RNase-free PCR tube:
   1ug of RNA sample
   49ul of 1X Reaction Buffer:
   1ul of DNase I, 1 unit/ml*
   * Referto the Certification of Analysis for the lot specific activity.
   To dissolve DNase I at a concentration of at least 1unit/ml, storage buffer is recommended: 50% Glycerol, 20mM Tris-HCl, pH 7.5, and 1 mM $MgCl_2$.
   This solution can be stored at -20°C for at least one year.
2. Incubate for 10 ~ 15 minutes at 37°C.
3. To stop the reaction, add 1ul of Stop Solution to bind calcium and magnesium ions and to inactivate the DNase I.

Note: The Stop Solution (20mM EDTA) must be added before heating to prevent metal (Mg/Ca) ion catalyzed hydrolysis of the RNA.
Heat at 70°C for 10 minutes to denature both the DNase I and the RNA. This product should not be used for digestions longer than 15 minutes or for digestions at temperatures higher than 37°C, or the residual contaminating RNase activity will begin to degrade the RNA.

Usage Note

1. This DNase solution does not contain an RNase inhibitor.
2. Under different buffer conditions the amount of DNase required to completely digest a given amount of DNA may need to be empirically determined. For example, salt concentrations >100mM will reduce DNase activity.

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