vivantis

PRODUCT INFORMATION
Deoxyribonuclease I (DNase I)

Product: Deoxyribonuclease I (DNase I)

Grade: Molecular Biology Grade

Code: PC0704-25mg / 50mg / 100mg

Molecular Weight: 31,000 Daltons

Lot No.:

Expiry Date:

Description

DNase I is an endonuclease derived from bovine pancreas that will degrade double-stranded DNA and produce 3'-OH oligonucleotides in the presence of divalent cations. In the reaction solution, with the presence of Mg^{2+} helps the enzyme to produce nicks in double-stranded DNA, while with the presence of Mn^{2+}, DNase I cleaves both strands of the DNA. DNase I is also 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 offered as lyophilized powder with an activity of 500 Kunitz U/mg and is a chromatographically pure preparation. For greatest stability, DNase I is suggested to be dissolved at a concentration of **at least 1mg/ml in 50% glycerol with 20mM Tris-HCl, pH7.5, and 1mM $MgCl_2$**. This solution can be stored at -20°C for at least a year.

Unit Definition

One unit of DNase I is defined as the amount required to completely degrade 1µg of lambda DNA in 10 minutes at 37°C in 50µl of a buffer containing 40mM Tris-HCl (pH8.0), 2.5mM (up to 10mM) MgSO4, 1mM (up to 10mM) CaCl2. Under these assay conditions one unit of DNase activity is approximately equal to one Kunitz unit.

Storage Temperature:

Store at -20°C. Avoid exposure to frequent temperature changes. See the expiration date on the stickers of product item.

Storage Buffer:

50% glycerol solution, 20mM Tris-HCl pH7.5, and 1mM $MgCl_2$.
Concentration of stock solutions will vary depending on application.

Recommended 1x Reaction Buffer

40mM Tris-HCl pH8.0, 2.5mM (up to 10mM) $MgSO_4$, 1mM (up to 10mM) $CaCl_2$
Note:
*If starting material contains EGTA, EDTA or other chelating reagents, and/or high concentration of salts, higher concentration of Mg and Ca are recommended.
**DNase I is sensitive to physical denaturation. Gently mix preparations by inversion; do not vortex.

Heat Inactivation

Incubate samples at 65°C for 10 minutes. To protect RNA from being degraded during DNase I inactivation, add EDTA to a final concentration of 5mM.

Requirement

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

Inhibitors

EGTA; EDTA; salt concentrations>100mM will reduce DNase activity

Stop Solution

20mM EGTA (pH8.0)

Procedure

1. For each 1µg of nucleic acid to be DNase I treated, prepare the reaction mix below:

Components	Volume
1X Reaction Buffer	49µL
DNase I Solution (1U/µL)	1µL
Nucleic Acid	1µg

2. Gently mix the reaction until the mixture is completely well mixed.
3. Incubate at 37°C for 10-15 minutes.
   *Incubation time may be increased to 30 minutes for removal of genomic DNA.
4. To stop the reaction, add 1µl of Stop Solution to bind calcium and magnesium ions and to inactivate the DNase I. Heat at 70°C for 10 minutes to denature both the DNase I and the RNA.
   *The Stop Solution (20mM EGTA) must be added before heating to prevent metal (Ca/Mg) ions catalyzed hydrolysis of the RNA. This product should not be used for digestion longer than 15 minutes or for digestion at temperature higher than 37°C, or the residual contaminating RNase activity will begin to degrade the RNA.

Usage Notes

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