PRODUCT INFORMATION

DNase I –DD0099 (D0099)

Product information for DD0099:

Description:
Bio Basic Inc's DNase I (catalogue DD0099) 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+} I 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.

Bio Basic Inc.'s DNase I is a chromatographically pure preparation. It is offered as lyophilized powder with an approximately activity of 500 Kunitz U/mg. For greatest stability, it is important that DNAse be dissolved at a concentration of at least 1mg/ml in 50% Glycerol with 20mM Tris-Cl, pH 7.5, and 1mM $MgCl_2$. This solution can be stored at -20ºC for at least a year.

Recommended 1X Reaction Buffer: 40mM Tris-HCl (pH 8.0), 2.5mM (up to 10mM) $MgSO_4$, 1mM (up to 10mM) $CaCl_2$
Note: If starting material contains EGTA, EDTA, other chelating reagents, and/or high concentration of salts, higher concentration of Mg and Ca are recommended.

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

Inhibitors: EGTA; EDTA (7); salt concentrations >100mM will reduce DNase activity.

Molecular Weight: 31,000 Daltons.

Requirement: Ca2+ and Mg2+ or Mn2+

Source: Bovine pancreas.

Storage Temperature: Store at –20°C. Avoid exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Stop Solution: 20mM EGTA (pH 8.0).

Unit Definition**:** One unit of DNase 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 (pH 8.0), 2.5mM (up to 10mM) $MgSO_4$, 1mM (up to 10mM) $CaCl_2$. Under these assay conditions one unit of DNase activity is approximately equal to one Kunitz unit. See the unit concentration on the Product Information Label.

20 Konrad Cres. Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

Procedure:

1. Add to an RNase-free PCR tube:
   - 1 µg of RNA sample
   - 49µl of 1X Reaction Buffer: 40mM Tris-HCl (pH 8.0), 2.5mM (up to 10mM) $MgSO_4$, 1mM (up to 10mM) $CaCl_2$
   - 1 µl of DNase I, 1 unit/ml*

*Refer to the Certificate 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-Cl, pH 7.5, and 1 mM $MgCl_2$. This solution can be stored at -20^{o}C for at least one year.

2. Incubate for 10~15 minutes at 37^{o}C
3. To stop the reaction, add 1 µl of Stop Solution to bind calcium and magnesium ions and to inactivate the DNase I.

Note:

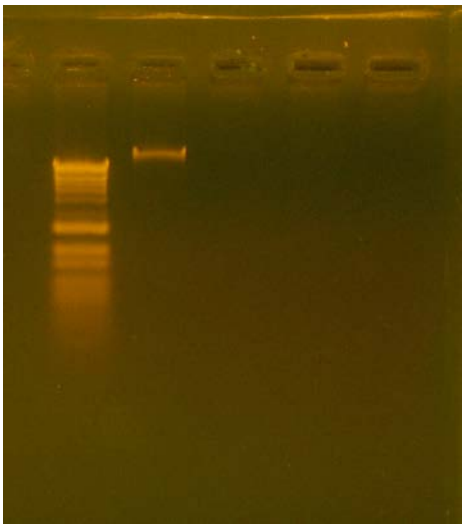
The Stop Solution (20 mM EGTA) must be added before heating to prevent metal (Mg/Ca) ion catalyzed hydrolysis of the RNA. Heat at 70 °C for 10 minutes to denature both the DNase I and the RNA.

This product should not be used for digestions longer than 15 minutes or for digestions at temperatures higher than 37^{o}C, or the residual contaminating RNase activity will begin to degrade the RNA.

Usage Notes:

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.

QC Results:



lane 1 lane 2 lane3 lane4 lane 5

Lane 1: Marker
Lane 2: Negative control (no DNase I)
Lane 3: DNA + 1 unit DNase I
Lane 4: DNA + 2 units DNase I
Lane 5: DNA + 3 units DNase I

20 Konrad Cres. Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com