



GIBCOBRL



LIFE TECHNOLOGIES™

## Deoxyribonuclease I, Amplification Grade

**Cat. No. 18068-015**

**Lot No.**

**Exp. Date:**

**Conc.: 100 Units, 1 U/μl**

**Store at -20°C in a non-frost-free freezer.**

### Description:

Deoxyribonuclease I, Amplification Grade (DNase I, Amp Grade) digests single- and double-stranded DNA to oligodeoxy-ribonucleotides containing a 5'-phosphate. It is suitable for eliminating DNA during critical RNA purification procedures such as those prior to RNA-PCR amplification. DNase I, Amp Grade is purified from bovine pancreas and has a specific activity of  $\geq 10,000$  U/mg.

### Components:

18068-015	DNase I, Amp Grade	Lot No.
Y02340	10X DNase I Reaction Buffer	Lot No.
Y02353	25 mM EDTA (pH 8.0)	Lot No.

### Unit Definition:

One unit increases the absorbance of a high molecular weight DNA solution at a rate of  $0.001 A_{260}$  units/min/ml of reaction mixture at 25°C.

### Storage Buffer:

20 mM sodium acetate (pH 6.5)  
5 mM CaCl<sub>2</sub>  
0.1 mM PMSF  
50% (v/v) Glycerol

### Unit Assay Conditions:

0.1 M sodium acetate (pH 5.0)  
5 mM MgCl<sub>2</sub>  
50 μg/ml calf thymus DNA

### 10X DNase I reaction Buffer:

200 mM Tris-HCl (pH 8.4), 20 mM MgCl<sub>2</sub>, 500 mM KCl

Doc. Rev.: 051497

This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Life Technologies TECH-LINE™ (800) 828-6686.

**Quality Control:**

The ability to digest double-stranded DNA to oligonucleotides is confirmed. DNase I and components are tested for the absence of RNase activity by incubating with 0.24-9.5 Kb RNA Ladder; gel analysis shows no degradation of the RNA ladder as compared to a no-DNase I control.

The enclosed buffers were assayed with the enzyme and met quality control specifications.

**Protocols:**

***Preparation of RNA Sample Prior to RT-PCR:***

Prepare duplicate tubes if positive and negative reverse transcriptase (RNA) samples are to be used in the amplification reaction. Add the following to an RNase-free, 0.5-ml microcentrifuge tube on ice:

- 1 µg RNA sample
- 1 µl 10X DNase I Reaction Buffer
- 1 µl DNase I, Amp Grade, 1 U/µl
- DEPC-treated water to 10 µl

**NOTE:** To work with larger amounts of RNA, scale up the reaction (including volume) linearly.

Incubate tube(s) for 15 min at room temperature. Inactivate the DNase I by the addition of 1 µl of 25 mM EDTA solution to the reaction mixture. Heat for 10 min at 65°C. The RNA sample is ready to use in reverse transcription, prior to amplification.

**NOTE:** It is important not to exceed the 15-minute incubation time or the room-temperature incubation. Higher temperatures and longer time could lead to Mg<sup>++</sup>-dependent hydrolysis of the RNA. Additionally, it is vital that the EDTA be added to at least 2 mM prior to heat-inactivation to avoid this problem.

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