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Deoxyribonuclease I, Amplification Grade

Cat. No. 18068-015 **Size: 100 Units**
Conc.: 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 ≥ 10,000 U/mg.

Components:
18068-015 DNase I, Amp Grade
Y02340 10X DNase I Reaction Buffer
Y02353 25 mM EDTA (pH 8.0)

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 $CaCl_2$
0.1 mM PMSF
50% (v/v) Glycerol

Unit Assay Conditions:
0.1 M sodium acetate (pH 5.0)
5 mM $MgCl_2$
50 µg/ml calf thymus DNA

10X DNase I reaction Buffer:
200 mM Tris-HCl (pH 8.4), 20 mM $MgCl_2$, 500 mM KCl

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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^{++}-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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