Deoxyribonuclease I, RNase free, 2 units/ul

D0200

Storage

Store at -20°C.

Do not store in a frost-free freezer.

Contents

- Product Manual
- Deoxyribonuclease I, RNase free, 2 units/ul
- 10X DNase I Buffer

Shipping Condition

Ship with dry ice.

Description

DNase I (RNase-free) is a high-purity DNase I for degradation of DNA in applica -tions where the absence of RNase is critical.

Background

Bovine pancreatic deoxyribonuclease I produced recombinantly in yeast. Pichia pastoris, to decrease levels of contaminating RNase and eliminate potential patho -gens associated with animal based materials. Bovine pancrease is a rich source of RNase A which is often found in many commerical DNase preparations. Pro -ducing DNase I by recombinant means in an organism with much lower levels of endogenous RNas greatly facilitates purification of an enzyme with undetected levels of recomninant DNase I are completely devoid of aminal based components which elimiates the possibility of introducing animal derived pathogens into bioprocessing procedures.

Source

Pichia pastoris

Unit Definition

One Unit causes an increase in absorbance at 260nm of 0.001 per minute at 25°C when acting upon highly polymerized DNA at ph 5.0.

Storage buffer

5mM calcium acetate, 4mg/ml glycine, pH 5.0 and 50% glycerol.

DNase I Reaction Buffer (10X)

500mM Tris-HCl, 10mM $MgSO_4$, 1mM $CaCl_2$, ph7.8, provided.

Application

DNase I (RNase-free) is used to degrade DNA in the presence of RNA when the absence of RNase is critical to maintain the integrity of the RNA. DNase I is fre -quently used to remove template DNA from in vitro transcription reactions (Krieg et al, 1985), or to detroy genomic DNA in RNA preparation prior to reverse transcription-PCR (RT-PCR)

Removal of contaminating genomic DNA from RNA samples

1. If the nucleic acid solution concentration is >200ug/mL, dilute it to 10ug nu -cleic acid/50 uL.
2. Add 10X DNase I Buffer (supplied) to 1X concentration in the RNA sample.
3. Add 1 uL DNase I (2U) for up to 10ug RNA in a 50uL reaction, and incubate at 37°C for 15 minutes.
4 Extract the RNA sample with phenol/chloroform to inactivate the DNase I.

Degradation of DNA template in a transcription reaction

1. After transcription, add 2U of DNase I to a 20 uL transcription reaction. It is not necessary to add 10X DNase I Buffer to the transcription reaction.
2. Incubate at 37°C for 15 minutes.

- If the transcript is to be gel purified, then gel loading buffer may be added directly to the DNase I-treated transcription reaction.
- If not, the DNase I can be inactivated by phenochloroform extraction.

Conditions for complete DNA digestion

1. Add 10X DNase I Buffer to 1X concentration in the solution to be DNase-treated, and add approximately 1-2U of DNase I per 1ug DNA present.
2. Incubate at 37°C for 15-30 minutes.

Heat inactivation of DNase I (RNase-free)

Some protocols suggest heating at 75°C for 5 minutes to inactivate DNase I (Huang, Fasco, and Kaminsky, 1996). We recommend a 10-minute incubation at 75°C for complete inactivation of DNase I (RNase-free) at a concentration of 0.1 U/UL. If this is the preferred method of inactivation, add EDTA to a final con -centration of 5mM before heating. If EDTA is not added, the RNA will undergo chemical scission when heated.

Note: For RNA sample that are to be used in reverse transcription reactions, the EDTA concentration in the RNA sample must be taken into account. Excess EDTA in an RNA sample may lower the free Mg2+ concentration and affect the efficiency of reverse transcription. After heat inactivation of DNase I. It may be necessary to add additional Mg2+ for maximum reverse transcriptase activity. Alternatively, DNase I can be inactivated and removed by phenol/chloroform extraction.

Gel analysis

Gel loading buffers should contain EDTA to eliminate DNase I activity: we reco -mmend using denaturing gel loading buffers chloroform extraction.

References

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Huang Z. Fasco MJ, and Kaminsky LS (1996) ***BioTechnique***s 20.1012-1020.

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