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DNase I (RNase free)

Catalog Number AM2222, AM2224

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Contents	Quantity	Storage conditions
DNase I , 2 U/µL	AM2222: 2000 Units AM2224: 5 X 2000 Units	Store at –20°C. *Do not store in a frost-free freezer.*
10X DNase I Buffer • 100 mM Tris, pH 7.5 • 25 mM $MgCl_2$ • 5 mM $CaCl_2$	1 mL	

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

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

Source: Bovine pancreas

Unit (U) definition: One unit is the amount of enzyme required to completely degrade 1 µg DNA in 10 min at 37°C, and it is equivalent to 0.04 Kunitz units.

Storage buffer (*not included*): 20 mM HEPES pH 7.5, 10 mM $CaCl_2$, 10 mM $MgCl_2$, 1 mM DTT and 50% (v/v) glycerol.

General information

DNase I (E.C. 3.1.21.1) from bovine pancreas is a 31-kDa glycoprotein. It is purified to be free of RNases and other contaminating proteins by an extensive series of chromatographic procedures. DNase I is an endonuclease that hydrolyzes phosphodiester linkages yielding oligonucleotides with a 5'-phosphate and a 3'-hydroxyl group (Kunitz, 1950). DNase I has been shown to act on single- and double-stranded DNA, chromatin, and RNA:DNA hybrids. DNase I requires bivalent cations (Mg^{2+} and Ca^{2+}) for maximal activity (Clark and Eichhorn, 1974; Junowicz and Spencer, 1973; Price, 1975).

Using DNase I (RNase-free)

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 Iis frequently used to remove template DNA from in vitro transcription reactions (Krieg et al, 1985), or to destroy genomic DNA in RNA preparations prior to reverse transcription-PCR (RT-PCR).

Removal of contaminating genomic DNA from RNA samples

1. If the nucleic acid solution concentration is >200 µg/mL, dilute it to 10 µg nucleic acid/50 µL.
2. Add 10X DNase I Buffer (supplied) to 1X concentration in the RNA sample.
3. Add 1 µL DNase I (2 U) for up to 10 µg RNA in a 50 µL reaction, and incubate at 37°C for 30 minutes.

   These reaction conditions will remove up to 2 µg of genomic DNA.
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 2 U of DNase I to a 20 µL 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 phenol-chloroform 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 1–2 U of DNase I per 1 µg 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/µL. If this is the preferred method of inactivation, add EDTA to a final concentration of 5 mM before heating. If EDTA is not added, the RNA will undergo chemical scission when heated.

Note: For RNA samples 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 Mg^{2+} concentration and affect the efficiency of reverse transcription. After heat inactivation of DNase I. It may be necessary to add additional Mg^{2+} 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 recommend using denaturing gel loading buffers such as Gel Loading Buffer II (Cat. no. AM8546G, AM8547).

Limited product warranty

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References

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