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Recombinant DNase I (rDNase I)

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Contents	Quantity	Storage conditions
rDNase I, 2 U/µL	1000 Units	Store at –20°C. *Do not store in a frost-free freezer.*
10X DNase I Buffer • 100 mM Tris-HCl, 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

Recombinant DNase I (rDNase I) is a highly purified DNase I derived from cloned bovine DNase I grown in a host with RNase levels that are 10^7-fold lower than bovine pancreas.

Source: A non-animal host that overexpresses bovine DNase I.

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

Recombinant DNase I (rDNase I) is a recombinant form of bovine DNase I purified from an overexpressing host. rDNase I is bovine-free. In addition, the host expressing rDNase I contains 10^7-fold less endogenous RNase activity than pancreatic tissues, the source for bovine DNase I. As a result, rDNase I can be more readily purified from contaminating RNases. The enzyme is provided free from any detectable RNase activity. rDNase I has a molecular weight of approximately 30 kDa.

DNase I is an endonuclease that hydrolyzes phosphodiester linkages yielding oligonucleotides with a 5'-phosphate and a free 3'-hydroxyl group (Kunitz, 1950). DNase I has been shown to act on single- and double-stranded DNA, chromatin, and RNA:DNA hybrids, although the specific activity of DNase I for RNA:DNA hybrids and single-stranded DNA is at least 2 orders of magnitude below that for double-stranded DNA. DNase I requires both Mg^{2+} and Ca^{2+} for maximal activity (Clark and Eichhorn, 1974; Junowicz and Spencer, 1973; Price, 1975).

Using rDNase I

The reaction kinetics of rDNase I are identical to that of native DNase I. Thus, rDNase I can be directly substituted for native DNase I with equivalent unit activity. Use rDNase I to degrade DNA in the presence of RNA when the absence of RNase is critical to maintain the integrity of the RNA. For example, DNase I is frequently used to remove template DNA after 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 rDNase 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 rDNase I.

Degradation of DNA template in a transcription reaction

1. After transcription, add 2 U of rDNase 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 rDNase I-treated transcription reaction.
   - If not, the rDNase 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 rDNase I per 1 µg DNA present.

2. Incubate at 37°C for 15–30 minutes.

Heat inactivation of rDNase I

Some protocols suggest heating at 75°C for 5 min to inactivate DNase I (Huang, Fasco, and Kaminsky, 1996). We recommend a 10-minute incubation at 75°C for complete inactivation of DNase I 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; denaturing gel loading buffers containing mostly formamide or formaldehyde, such as Gel Loading Buffer II (Cat. no. AM8546G, AM8547) will also inactive DNase I.

Limited product warranty

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References

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