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TURBO™ DNase

Catalog Number AM2238, AM2239

Pub. No. 4393900 **Rev.** B

Contents	Quantity	Storage conditions
TURBO™ DNase, 2 Units/µL	AM2238: 1000 Units AM2239: 5000 Units	Store at –20°C. *Do not store in a frost-free freezer.*
10X TURBO™ DNase Buffer	2 X 1.75 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

TURBO™ DNase is a genetically engineered form of bovine DNase I with greater catalytic efficiency than conventional DNase I at higher salt concentrations and lower DNA concentrations.

Source: A non-animal host that overexpresses mutant, 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

DNase I (E.C.3.1.21.1) is an endonuclease that hydrolyzes phosphodiester linkages yielding oligonucleotides with a 5' phosphate and a free 3' hydroxyl (Kunitz, 1950) group. DNase I has been shown to act on single- and double-stranded DNA, chromatin, and RNA:DNA hybrids, although the specific activity of DNase for RNA:DNA hybrids and single-stranded DNA is at least 2 orders of magnitude below that for double-stranded DNA. DNase I requires bivalent cations (Mg^{2+} and Ca^{2+}) for maximal activity (Clark and Eichhorn, 1974; Price, 1975).

TURBO™ DNase was developed using a protein engineering approach that introduced amino acid changes into the DNA binding pocket of wild-type DNase I. These changes markedly increase the affinity of the protein for DNA, with 6-fold lower K_m for DNA. Conventional DNase I is very sensitive to monovalent salt, and it cleaves DNA of low concentration very inefficiently. In contrast, TURBO™ DNase maintains at least 50% of peak activity in solutions with 200 mM monovalent salt, even when the DNA concentration is in the nanomolar (nM) range. TURBO™ DNase is up to 50-fold more active and has 350% greater catalytic efficiency than wild-type DNase I.

The proficiency of TURBO™ DNase in binding low-concentration DNA suggests that the enzyme is particularly effective in removing trace quantities of DNA contamination. This becomes important for complete removal of DNA from a sample, since the cleavable DNA substrate is reduced as the DNase reaction proceeds. As a result, TURBO™ DNase is more effective than wild-type DNase I in reducing DNA detection by PCR.

Using TURBO™ DNase

TURBO™ DNase is used to degrade DNA in the presence of RNA when the absence of RNase is critical to maintain the integrity of the RNA. For example, TURBO™ DNase can be used to eliminate plasmid DNA templates from *in vitro* transcription reactions, or to destroy genomic DNA in RNA preparations prior to reverse transcription-PCR (RT-PCR).

Note: Use TURBO™ DNase with the supplied digestion buffer. We do not recommend using TURBO™ DNase with DNase I Buffer (Cat. no. AM8170G) or another manufacturer's DNase I Buffer.

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 TURBO™ DNase Buffer (supplied) to 1X concentration in the RNA sample.
3. Add 1 µL TURBO™ DNase (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 TURBO™ DNase.

Degradation of DNA template in a transcription reaction

1. After transcription, add 2 U of TURBO™ DNase to a 20 µL transcription reaction. It is not necessary to add 10X TURBO™ DNase 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 TURBO™ DNase-treated transcription reaction.
   - If not, the TURBO™ DNase can be inactivated by phenol-chloroform extraction.

Conditions for complete DNA digestion

1. Add 10X TURBO™ DNase Buffer to 1X concentration in the solution to be DNase-treated, and add approximately 1–2 U of TURBO™ DNase per 1 µg DNA present.

2. Incubate at 37°C for 30 minutes.

Inactivation of TURBO™ DNase

Inactivate TURBO™ DNase using one of the following methods:

- (*Recommended*) Perform a phenol/chloroform extraction.
- Add EDTA to a final concentration of 15 mM, and heat at 75°C for 10 minutes (this inactivates >99% of TURBO™ DNase). 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, excess EDTA in an RNA sample may lower the free Mg^{2+} concentration and affect the efficiency of reverse transcription. After heat inactivation of TURBO™ DNase, it may be necessary to add additional Mg^{2+} for maximum reverse transcriptase activity.

(*Optional*) For best results when using RNA samples in downstream reactions, we recommend that you use the TURBO DNA-*free*™ Kit (Cat. no. AM1907), which contains both TURBO™ DNase and a specially formulated DNase Inactivation Reagent for removal of TURBO™ DNase from your RNA samples.

Gel analysis

Gel loading buffers should contain EDTA to eliminate TURBO™ DNase 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

Clark, R. and Eichhorn, GL. (1974) *Biochem* 13, 5098.

Kunitz, M. (1950) *J Gen Physiol* 33, 349.

Price, P.A. (1975) *J Biol Chem* 250,1981–1986.