Deoxyribonuclease I (DNase I)

RNase-free

Cat. No.	size
E1345-01	1 000 units
E1345-02	5 000 units

Nonspecific deoxyribonuclease that degrades both double-stranded and single-stranded DNA endonucleolytically releasing 5'-phosphorylated di-, tri-, and oligonucleotide products (2).

Applications:

- Preparation of DNA-free RNA (degradation of contaminating DNA after RNA isolation) (3).
- Preparation of DNA–free RNA prior to RT-PCR and RT-qPCR (4).
- Removal of template DNA following *in vitro* transcription.
- Studies of DNA-protein interactions (footprinting).
- DNA labeling by nick-translation.
- Production of random fragments (generation of libraries) (5).

Unit Definition:

One unit is the amount of enzyme required to completely degrade 1 µg of plasmid DNA in 10 min at 37°C. One functional DNase I unit is approximately equivalent to 0.3 Kunitz unit (1).

Enzyme activity:

DNase I requires Ca^{2+} and Mg^{2+} for hydrolyzing double-stranded DNA. In the presence of $Mg^{2+,}$ DNase I cleaves each strand of double-stranded DNA independently in a statistically random fashion (recommended Reaction Buffer I). In the presence of Mn^{2+}, the enzyme cleaves both DNA strands at approximately the same site, producing DNA fragments with blunt-ends or with overhang termini of only one or two nucleotide (recommended Reaction Buffer II) (5).

Storage Conditions:

Store at -20°C.

10 x Reaction Buffer I:

100 mM Tris-HCl, 25 mM $MgCl_2$, 100 mM $CaCl_2$, pH 7.4 at 25°C.

10 x Reaction Buffer II:

100 mM Tris-HCl, 100 mM $CaCl_2$, pH 7.4 at 25°C. Supplemented with 100 mM $MnCl_2$.

Inactivation:

Inactivated by heating at 65°C for 10 min in the presence of EDTA or EGTA.

Inhibitors:

Metal chelators (EGTA, EDTA), transition metals, SDS, reducing agents (DTT, β-mercaptoethanol).

Quality Control:

Functionally tested for digesting of template-plasmid DNA. The absence of RNase confirmed by appropriate quality test utilizing spectrophotometry assays of RNA sample concentration before and after incubation with an excess of enzyme.

Note 1: This DNase solution does not contain an RNase inhibitor. Please handle with care to avoid contaminating it with RNase.

Note 2: DNase I is sensitive to physical denaturation. Therefore, do not vortex solutions containing DNase I, mix by gently flipping the tube or pipetting.

References:

1. *Kunitz, M (1950) J. Gen Physiol 33: 349-362.*
2. *Vanecko, S and Laskowski, M (1961). J Biol Chem 236: 3312-3316.*
3. *Although it is not required for most applications please see the additional DNase I digestion conditions in Manual for GeneMATRIX UNIVERSAL DNA/RNA/ Protein Purification Kit (E3597) and GeneMATRIX UNIVERSAL RNA Purification Kit (E3598).*
4. *Sanyal, A., et al., An effective method of completely removing contaminating genomic DNA from an RNA sample to be used for PCR. Mol. Biotechnol., 8, 135-137, (1997).*
5. *Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.*

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Protocol - RNA isolation: Complete digestion of DNA

1. Mix:

Component:	Amount:
RNA in aqueous solution (e.g. H_2O, 1 x TE)	1-8 µl
RNase-free water (e.g. EURx Cat.No. E0210)	0-7 µl
10 x Reaction Buffer I*	1 µl
RNase-free DNase I	1 U per 1 µg RNA
Total reaction voulme	10 µl

*Use Reaction Buffer I for complete DNA digestion and Reaction Buffer II for generation of randomized DNA libraries.

2. Incubate at 37°C for 15-30 min (or shorter for random partial digestion of DNA to larger fragments).
3. Stop reaction and inactivate DNase I by alternatively performing:
    - Heat inactivation (65°C, 10 min) in presence of 20 mM EDTA, or
    - Spin Column RNA Purification (e.g. EURx Universal RNA Purification Kit, Cat.No. E3598), or
    - Proteinase K digestion, followed by acidic phenol-chloroform extraction (at pH 4.5, residual DNA will remain with the interphase), or
    - Lithium chloride precipitation (selectively precipitates RNA; residual DNA remains in supernatant).

On-column digestion (RNA purification kits)

1. EURx DNase I is compatible with all commonly used RNA kits supplied by third parties.
2. Use ~1 U of DNase I (RNase free) per column.
3. Refer to the detailed instructions in the manual accompanying the RNA kit.
4. In general, on column DNase digestion is known to proceed with reduced efficiency as compared to DNase digestion in aqueous solution. DNase may not obtain optimal steric access to column-bound DNA.
5. The fastest, most straightforward, most gentle and least expensive approach to RNA isolation is, of course, not to use DNase I at all: DNase digestion is mostly not required with EURx RNA spin column kit series (e.g. EURx Universal RNA Purification Kit, Cat.No. E3598).

Protocol - Random partial digestion of DNA in presence of Mn^{2+} ions (5)

1. Aliquot 5 µl of 50 mM EDTA (pH 8.0) into each of six plastic reaction tubes. Keep on ice.
2. Dilute 1 U of DNaseI in 100 µl ice cold 1 x Reaction Buffer II (containing Mn^{2+} ions). Keep on ice.
3. Starting with 5-10 µg of target DNA, assemble the following reaction mixture:

Component:	Amount:
Target DNA [200-400 µg/ml]	25 µl
10 x Reaction Buffer II	10 µl
Nuclease–free Water	65 µl

Zero control: Transfer 10 µl of reaction to one of the tubes containing 5 µl 50 mM EDTA prior to addition of DNase.

4. To the remainder of the mixture add 1.5 µl of diluted DNase I.
5. Incubate at 15°C.
6. *Time series:* After 1, 2, 5, 10 and 30 min of incubation, respectively, transfer 15 µl of the reaction to one of the prepared tubes containing 5 µl 50 mM EDTA (total volume: 20 µl each tube). Mix completely by gentle pipetting. Keep all tubes on ice until sample collection is complete.
7. Analyze aliquots of digested DNA (2-3 µl each) on a 1.5% agarose gel.
8. Pool those samples that do contain DNA fragments in the size range of interest (e.g. 0.5 to 1.5 kb).
9. Purify pooled DNA with EURx PCR /DNA Clean-Up Purification Kit (Cat. No. E3520), or with EURx 3 in 1 Basic DNA Purification Kit (Cat. No. E3545), or by phenol-chloroform extraction.
10. Convert DNA ends to blunt ends by polishing DNA with a mixture of Klenow Fragment *exo*$^-$ (Cat No. E1092) and T4 DNA Polymerase (Cat No. E1100). Using both polymerases simultaneously for blunting DNA ends enhances efficiency of the reaction and has beneficial effects on further reaction steps and on subsequent cloning.

Note: Prior to digestion of precious sample DNA, evaluate and adjust the proper reaction conditions using less important test DNA.