

DNase I, RNase-free

89836

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Number

89836

Description**DNase I, RNase-free**, 1000 units (1 unit/ μ L)Storage Buffer: 50mM Tris•HCl (pH 7.5), 10mM CaCl₂, 50% glycerol

Molecular Weight: ~29,000Da

Source: *E. coli* containing a cloned gene encoding bovine DNase IActivity: 1 unit of enzyme completely degrades 1 μ g of plasmid DNA in 10 minutes at 37°C

Supplied with:

10X Reaction Buffer (1mL): 100mM Tris-HCl (pH 7.5), 25mM MgCl₂, 1mM CaCl₂

50mM EDTA (1mL)

Storage: Upon receipt store at -20°C. Product is shipped with dry ice.**Introduction**

Thermo Scientific™ DNase I is commonly used to degrade unwanted single- and double-stranded DNA into 5' phosphodinucleotide and oligonucleotide fragments.¹ The properties of DNase I can be modified by divalent ions. For example, in the presence of Mg²⁺, Ca²⁺ or Zn²⁺, DNase I degrades DNA by making random single-strand nicks in the phosphate backbone.² In the presence of divalent transition metals such as Mn²⁺ or Co²⁺, DNase I creates double-strand breaks, resulting in fragments with 0-2 nucleotide overhangs.² DNase I is suitable for removal of genomic DNA from cell lysates, removal of plasmid from *in vitro* transcribed RNA,³ nick translation^{4,5} and DNase I footprinting.⁶

Important Product Information

- Avoid storing DNase I in frost-free freezers, as temperature fluctuations will reduce its activity. Maintain DNase I on ice until ready to use.
- DNase I activity is influenced by divalent ions. DNase I has 10 times greater activity in buffer containing both 5mM Mg²⁺ and 130 μ M Ca²⁺ than either metal alone.⁷
- Calcium is required to maintain structure and activity of DNase I.¹ Trace amounts of Ca²⁺ may be present at sufficient concentration for DNase I to be active, but using calcium-free buffers or removal of Ca²⁺ by adding EGTA can reduce DNase I activity to undetectable levels.^{1,8}
- DNase I is inhibited by metal chelators, monovalent metal ions such as Na and K (i.e., \geq 100mM NaCl), SDS even at concentrations below 0.1%, reducing agents and ionic strength above 50-100mM.
- DNase I is inactivated by heating to 65°C for 10 minutes in the presence of EGTA or EDTA (use at least 1 mole of EGTA or EDTA per 1 mole of Mn²⁺/Mg²⁺).⁹
- DNase I is sensitive to physical denaturation. Mix gently by inverting tube. Do not vortex.

Procedures for Using DNase I with Protein Extracts

When using Thermo Scientific™ B-PER™ Reagent (Product No. 78248) or other lysis buffer to extract proteins, it may be necessary to degrade the genomic and plasmid DNA with DNase I to facilitate downstream applications. Trace amounts of Mg²⁺ and Ca²⁺ may be present in bacterial extracts. However, if optimal activity is required or if DNase I activity is low, supplement the lysis buffer with divalent ion(s) (see Important Product Information Section) before adding DNase I.

Note: The following protocols are examples and intended as a guide for using this product. Specific applications may require optimization.

Protocol 1: Removing Genomic DNA

A. Method

1. Equilibrate the protein extract to room temperature.
2. If desired, add 100µL of 10X Reaction Buffer per milliliter of extract and mix well.
3. Add 5-50µL of DNase I (5-50 units) per milliliter of extract and invert tube to mix (do not vortex).
Note: Up to ~250 units of DNase I may be used depending on the application. Adjust the amount of DNase I as needed.
4. Incubate reaction at 37°C for 30-60 minutes or until viscosity is sufficiently reduced.
5. If desired, inactivate DNase I by adding 100µL of 50mM EDTA per milliliter of extract, mix well and heat to 65°C for 10 minutes.
Note: Chelators, such as EDTA and EGTA, are not compatible with 6xHis-protein purification on nickel- or cobalt-chelated agarose.

Protocol 2: Removing Genomic DNA and Cell Wall Material from Inclusion Bodies

A. Optional Materials

- Triton™ X-100 Detergent (Product No. 28314) or other non-ionic detergent diluted to 0.5-1% with phosphate-buffered saline (PBS) or Tris-EDTA buffer
- Lysozyme (Product No. 89834) dissolved in B-PER Reagent or other suitable buffer at 10mg/mL
- Thermo Scientific™ Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X) (Product No. 87785)
- Inclusion body solubilization buffers

B. Method

1. Prepare a bacterial cell extract using a mechanical or reagent based lysis method, such as B-PER Bacterial Protein Extraction Reagent (Product No. 78243). Ensure lysis is sufficient to significantly disrupt cell wall and release inclusion bodies. If desired, add Halt Protease Inhibitor Cocktail, EDTA-free (Product No. 87785) to the extract to help preserve the sample.
2. Equilibrate the bacterial cell extract containing inclusion bodies to room temperature.
3. (Optional) To ensure proper DNase activity, add 100µL of 10X Reaction Buffer per milliliter of extract and mix well.
4. Add 5-50µL of DNase I (5-50 units) per milliliter of extract to degrade DNA. Mix by inversion (do not vortex). Incubate reaction at 37°C for 30-60 minutes or until viscosity is sufficiently reduced.
Note: Up to ~250 units of DNase I may be used depending on the application. Adjust the amount of DNase I as needed. Do not vortex or vigorously mix DNase I.
5. To degrade cell wall debris, add 20µL of Lysozyme stock for each milliliter of extract to a final concentration of 200µg/mL. Vortex tube for 1 minute.
Note: Lysozyme and DNase I may be added at the same time if desired. However, for greatest Lysozyme efficiency, the inclusion body pellets must be mixed well. Vortexing will not reduce Lysozyme activity.
6. To collect inclusion bodies, centrifuge tube at 12,000 × g for 10 minutes and remove the supernatant.

7. To wash and remove membrane contaminants from inclusion bodies, suspend the inclusion body pellet in a solution of 0.5-1% Triton X-100 Detergent (or alternative non-ionic detergent) in PBS. Vortex for 1 minute.
8. Repeat Steps 6-7 two more times.
9. Dissolve the purified inclusion bodies in denaturing agents and proceed to further refolding or purification procedures.

Additional Information

Please visit our website for additional information relating to this product including the following items:

- Tech Tip #43: Protein stability and storage
- Tech Tip #20: Dialysis: an overview
- Tech Tip #6: Extinction coefficients guide

Related Thermo Scientific Products

88700-2	Pierce™ Universal Nuclease for Cell Lysis
78243	B-PER Bacterial Protein Extraction Reagent, 165mL
78260	B-PER II Bacterial Protein Extraction Reagent, 250mL
78266	B-PER Reagent (in Phosphate Buffer), 500mL
88227-8	HisPur™ Ni-NTA Spin Purification Kit
16106-7	Pierce GST Spin Purification Kit
89833-4	Lysozyme
78115	Inclusion Body Solubilization Reagent, 100mL

Cited References

1. Moore, S. (1981). Pancreatic DNase. In: *The Enzymes*. Boyer, P.D., eds. New York: Academic Press. pp. 281-96.
2. Campbell, V.W. and Jackson, D. A. (1980). The effect of divalent cations on the mode of action of DNase I. *J Biol Chem* **255(8)**:3726-35.
3. Krieg, P.A. and Melton, D.A. (1987). *In vitro* RNA synthesis with SP6 RNA polymerase. *Methods in Enzymology, Recombinant DNA*, ed by R. Wu. Part F. **155**:397-415.
4. Sambrook, J., *et al.*, (1989). *Molecular Cloning: A Laboratory Manual*, 2nd ed. New York: Cold Spring Harbor Laboratory Press. 10.6-10.12.
5. Tabor, S., *et al.* (1997). DNA-Dependent DNA Polymerases. In: *Current Protocols in Molecular Biology*. Ausubel *et al.*, eds. Wiley & Sons Inc. 3.5.4-6.
6. Vitolo, J.M., *et al.* (1999). DNase I and hydroxyl radical characterization of chromatin complexes. In: *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds. Wiley & Sons Inc. 21.4.1-6.
7. Melgar, E., and Goldthwait, D.A. (1968). Deoxyribonucleic acid nucleases II. The effects of metal on the mechanism of action of deoxyribonuclease I. *J Biol Chem* **243(17)**:4409-16.
8. Pan, C.Q. and Lazarus, R.A. (1999). Ca²⁺-dependent activity of human DNase I and its hyperactive variants. *Protein Sci* **8(9)**:1780-8.
9. Woame, I., *et al.* (2000). Irreversible heat inactivation of DNase I without RNA degradation. *BioTechniques* **29**: 252-6.

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