

89835

1739.0

Number	Description
89835	DNase I , 5,000 units (5,000-10,000 units/ml) Storage Buffer: 10 mM Tris•HCl (pH 7.5), 10 mM CaCl ₂ , 10 mM MgCl ₂ , 50% glycerol Molecular Weight: ~36,000 Source: Bovine pancreas Activity: ≥ 100,000 units/mg Storage: Upon receipt store at -20°C. Product is shipped with dry ice.

Introduction

DNase I is a glycosylated polypeptide that 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. In a buffer at pH 7.2 containing 50 mM Tris•HCl, DNase I has high activity in the presence of either 6.6 mM Ni²⁺, 5.2 mM Mg²⁺, 660 μM Mn²⁺, 660 μM Co²⁺, 660 μM Ba²⁺, 660 μM Sr²⁺, 130 μM Ca²⁺ or 130 μM Zn²⁺. DNase I has 10 times greater activity in buffer containing both 5 mM Mg²⁺ and 130 μM 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 high enough 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}
- Monovalent metal ions such as Na⁺ and K⁺ (i.e., ≥ 100 mM NaCl) will decrease DNase I activity.⁷
- DNase I is inactivated by heating to 65°C for 10 minutes.

Procedures for Using Dnase I with Protein Extracts

When using 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.

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. Optional Materials

- 100X Reaction Buffer: 100 mM Tris•HCl (pH 7.5), 500 mM MgCl₂, 13 mM CaCl₂
- 10X Stop Solution: 200 mM EGTA or 500 mM EDTA

B. Method

1. Equilibrate the protein extract to room temperature.
2. If desired, add 10 µl of 100X Reaction Buffer per milliliter of extract and mix well.
3. Add 2-10 µl of DNase I (10-100 units) per milliliter of extract and invert tube to mix (do not vortex).

Note: Up to 25 µg/ml (~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 100 µl of Stop Solution per milliliter of extract and mix well.

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

- B-PER[®] Bacterial Protein Extraction Reagent, or other lysis buffer, diluted 1/10 with PBS or Tris-EDTA buffer
- 100X Reaction Buffer: 100 mM Tris•HCl (pH 7.5), 500 mM MgCl₂, 13 mM CaCl₂
- Lysozyme (Product No. 89834) dissolved in B-PER[®] Reagent, or other suitable buffer, at 10 mg/ml

B. Method

1. Equilibrate the protein extract containing inclusion bodies to room temperature.
2. If desired, add 10 µl of 100X Reaction Buffer per milliliter of extract and mix well.
3. Add 2-10 µl of DNase I (10-100 units) per milliliter of extract and mix by inversion (do not vortex).

Note: Up to 25 µg/ml (~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.

4. Incubate reaction at 37°C for 30-60 minutes or until viscosity is sufficiently reduced.
5. Add 20 µl of Lysozyme per milliliter of the reaction for a final concentration of 200 µg/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. Resuspend the inclusion body pellet in the diluted B-PER[®] Reagent and 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 the Pierce web site for additional information relating to this product including the following items:

- Tech Tip: Protein stability and storage
- Tech Tip: An overview of dialysis
- Tech Tip: Extinction coefficients guide

Related Pierce Products

78248	B-PER[®] Bacterial Protein Extraction Reagent, 500 ml
78243	B-PER[®] Bacterial Protein Extraction Reagent, 165 ml
78260	B-PER[®] II Bacterial Protein Extraction Reagent, 250 ml
78266	B-PER[®] Reagent (in Phosphate Buffer), 500 ml
78100	B-PER[®] 6xHis Fusion Protein Column Purification Kit
78300	B-PER[®] 6xHis Fusion Protein Spin Purification Kit
78200	B-PER[®] GST Protein Column Purification Kit
78400	B-PER[®] GST Fusion Protein Spin Purification Kit
78510	T-PER[®] Tissue Protein Extraction Reagent, 500 ml
89833	Lysozyme, 5 g
89834	Lysozyme, 25 g
78115	Inclusion Body Solubilization Reagent, 100 ml

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Product References

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B-PER[®] Technology is protected by U.S. Patent #6,174,704.

The most current versions of all product instructions are available at www.piercenet.com. For a faxed copy, contact customer service (in the USA call 800-874-3723) or your local distributor.

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