

Benzonase[®] Nuclease

Description

Benzonase Nuclease, Purity >99%	10 KU	70664-3
Benzonase Nuclease HC, Purity >99%	25 KU	71206-3
Benzonase Nuclease, Purity >90%	10 KU 2.5 KU	70746-3 70746-4
Benzonase Nuclease HC, Purity >90%	25 KU	71205-3

Benzonase is a genetically engineered endonuclease from *Serratia marcescens* (1–2). The enzyme is produced and purified from *E. coli* strain W3110, a mutant of strain K12, containing the proprietary pNUC1 production plasmid (3–4). Structurally, the protein is a dimer of identical 245 amino acid, ~30 kDa subunits with two essential disulfide bonds (5–8). This promiscuous endonuclease attacks and degrades all forms of DNA and RNA (single stranded, double stranded, linear and circular) and is effective over a wide range of operating conditions (9). The enzyme completely digests nucleic acids to 5'-monophosphate terminated oligonucleotides 2–5 bases in length (2, 10). Although the nuclease is capable of cleavage at nearly all positions along a nucleic acid chain, sequence-dependent preferences have been demonstrated (11). The enzyme prefers GC-rich regions in dsDNA while avoiding d(A)/d(T)-tracts. Benzonase is available from Merck KGaA, Darmstadt, Germany in two grades, Purity > 99% and Purity > 90%. Both preparations possess exceptionally high specific activities and are supplied free from measurable protease activities and viral contaminants. Both grades are available at 25–29 U/μl or at a high concentration (HC) of 250 U/μl. Benzonase is ideal for a wide variety of applications where complete digestion of nucleic acids is desirable.

Unit definition

One unit of Benzonase Nuclease is defined as the amount of enzyme that causes a ΔA_{260} of 1.0 in 30 min, which corresponds to complete digestion of 37 μg of DNA. Note that 1 KU = 1000 units.

Storage

Benzonase Nuclease is supplied in 50% glycerol containing 20 mM Tris-HCl pH 8.0, 20 mM NaCl and 2 mM MgCl₂. The enzyme preparation is stable for 2 years when stored at –20°C.

DO NOT store at –70°C because freezing Benzonase Nuclease results in loss of activity.

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Using Benzonase® Nuclease

Applications

- Viscosity reduction in protein extracts
- Sample preparation for 2D gel electrophoresis
- Removal of nucleic acid contaminants from recombinant protein preparations

Notes:

a) *Benzonase retains its activity under a wide range of conditions as follows.*

Condition	Optimal	Effective
Mg ²⁺ concentration (see note d below)	1–2 mM	1–10 mM
pH	8.0–9.0	6.0–10.0
Temperature	37°C	0–42°C
Dithiothreitol	0–100 mM	> 100 mM
2-mercaptoethanol	0–100 mM	> 100 mM
Monovalent cation concentration (Na ⁺ , K ⁺ , etc.)	0–20 mM	0–150 mM

- b) *Benzonase is inhibited (approximately 50% reduction in relative activity) by monovalent cation concentrations > 50 mM, phosphate concentrations > 20 mM, and by ammonium sulfate concentrations > 25 mM.*
- c) *Benzonase can be diluted for ease of handling small quantities with 50 mM Tris-HCl, 20 mM NaCl, 2 mM MgCl₂, pH 8.0. Diluted samples can be stored at 4°C for several days without loss of activity.*
- d) *Although Benzonase requires Mg²⁺ for activation, it does not appear to require additional Mg²⁺ under many conditions.*
- e) *Benzonase treatment is not generally recommended for purification of proteins that must be nuclease free. However, depending on the processing methods, Benzonase may be removed during purification. Residual nuclease activity can be checked by incubation of the purified protein with RNA or DNA markers followed by gel analysis.*
- f) *Benzonase is compatible with BugBuster® Protein Extraction Reagent and PopCulture® Reagent and may be combined with these reagents prior to use (see next page). Benzonase appears to have no effect on the FRETWorks™ S•Tag™ Assay.*

Examples

The following examples illustrate the use of Benzonase Nuclease, and are intended as guidelines only. Optimal conditions will vary from application to application, depending on buffer compositions, sample properties and reaction conditions.

Viscosity reduction in *E. coli* lysates

7.5 g *E. coli* W3110 (wet weight) were resuspended in 15 ml of 10 mM Tris-HCl, 1 mM EDTA, pH 9.0. MgCl₂ was added to a final concentration of 6 mM. 5 ml aliquots were taken from the suspension, and Benzonase Nuclease Purity > 90% was added at increasing concentrations.

The aliquots were passed through a French press at 10,000 psi and immediately placed at 0°C. Viscosity was judged at various time intervals by dripping from a pipet tip.

Benzonase Nuclease concentration	Incubation time to obtain “aqueous” drops
0.24 U/ml	> 60 min
2.40 U/ml	15 min
8.0 U/ml	5 min
24.0 U/ml	1.25 min

Removal of nucleic acids

A solution of herring sperm DNA was made in 50 mM Tris-HCl, 1 mM MgCl₂, 0.1 mg/ml BSA, pH 8.0. Samples were treated with various concentrations of Benzonase® at 0°C, 23°C or 37°C. At increasing time intervals, 10 µl aliquots (initially containing 500 ng DNA) were removed and applied to nitrocellulose membrane. The blot was probed with ³²P-labeled herring sperm DNA under low stringency hybridization and washing conditions to optimize detection of repetitive sequences in the herring sperm DNA. DNA standards from 100 ng down to 10 pg allowed semi-quantitative evaluation of residual hybridizable DNA.

Residual hybridizable DNA (in ng) after increasing incubation time

Benzonase Nuclease concentration	0 h	4 h	6 h	22 h
90 U/ml	500 ng	0.2	0.02	none*
9 U/ml	500	5	2	0.3

*not detectable after 30 h exposure

Residual hybridizable DNA (in ng) after increasing incubation time with 90 U/ml

Incubation temperature	0 h	4 h	6 h	22 h	30 h
37°C	500 ng	0.20	0.02	none*	none*
23°C	500	0.5	0.100	0.01	none*
0°C	500	1	0.500	0.05	0.01

*not detectable after 30 h exposure

Residual hybridizable DNA (in ng) after increasing incubation time with 90 U/ml

Buffer	0 h	4 h	6 h	22 h	30 h
Tris buffer (23°C)	500 ng	0.5	0.1	none*	none*
PBS buffer (23°C)	500	5	1	0.50	0.3

*not detectable after 30 h exposure

Use in conjunction with BugBuster® Protein Extraction Reagent

Benzonase Nuclease is ideal for use in combination with BugBuster Reagent (Cat. No. 70584-3) for rapid, convenient preparation of *E. coli* protein extracts (see Technical Bulletin 245). Cell extracts prepared with BugBuster can be viscous due to high molecular weight genomic DNA and other nucleic acids. The addition of 25 U Benzonase per 1 ml BugBuster greatly reduces the viscosity of the extract during a 10–20 minute incubation at room temperature. No additives are required; Benzonase remains fully active in 1X BugBuster Reagent for weeks at 4°C (unpublished results). Benzonase-treated extracts produce excellent flow rates on chromatography columns, and purification runs can be performed in much less time than untreated extracts.

Notes: To further enhance the effectiveness of cell lysis and protein extract preparation rLysozyme™ Solution (Cat. No. 71110-3) may be added with BugBuster Reagent. Lysozyme cleaves a peptidoglycan bond in the E. coli cell wall, enhancing cell lysis and increasing the yield of protein. Extract from hosts expressing T7 lysozyme (e.g. pLysS host) does not require exogenous lysozyme treatment.

Alternatively, Lysonase™ Bioprocessing Reagent (Cat. No. 71230), an optimized ready to use mix of rLysozyme Solution and Benzonase Nuclease can be used instead of separate additions of Benzonase and rLysozyme. For efficient protein extraction with BugBuster use 10 µl Lysonase per gram wet cell paste.

Use in conjunction with PopCulture® Reagent

Benzonase Nuclease is ideal for use in combination with PopCulture Reagent (Cat. No. 71092-3) for preparation of *E. coli* protein extracts directly in their culture medium without cell harvest, mechanical disruption or extract clarification (see Technical Bulletins 323 and 327) during a 10–15 min incubation. Benzonase (25 U per 1 ml original culture volume) can be pre-mixed with PopCulture to facilitate rapid sample processing and greatly reduces viscosity due to high molecular weight genomic DNA and other nucleic acids in the extract. Proteins in this total culture extract can be directly screened or purified by adding an affinity matrix. Benzonase-treated extracts produce excellent flow rates on chromatography columns, and purification runs can be performed in much less time than untreated extracts.

Notes: To further enhance the effectiveness of cell lysis and protein extract preparation rLysozyme™ Solution (Cat. No. 71110-3) may be added with PopCulture® Reagent and Benzonase. Lysozyme cleaves a peptidoglycan bond in the E. coli cell wall, enhancing cell lysis and increasing the yield of protein. Extracts from hosts expressing T7 lysozyme (e.g. pLysS host) does not require exogenous lysozyme treatment.

Alternatively, Lysonase™ Bioprocessing Reagent (Cat. No. 71230), an optimized ready to use mix of rLysozyme Solution and Benzonase Nuclease can be used instead of separate additions of Benzonase and rLysozyme. For efficient protein extraction with PopCulture use 2 µl per 1 ml extract.

References

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