EMD 4 BIOSCIENCES
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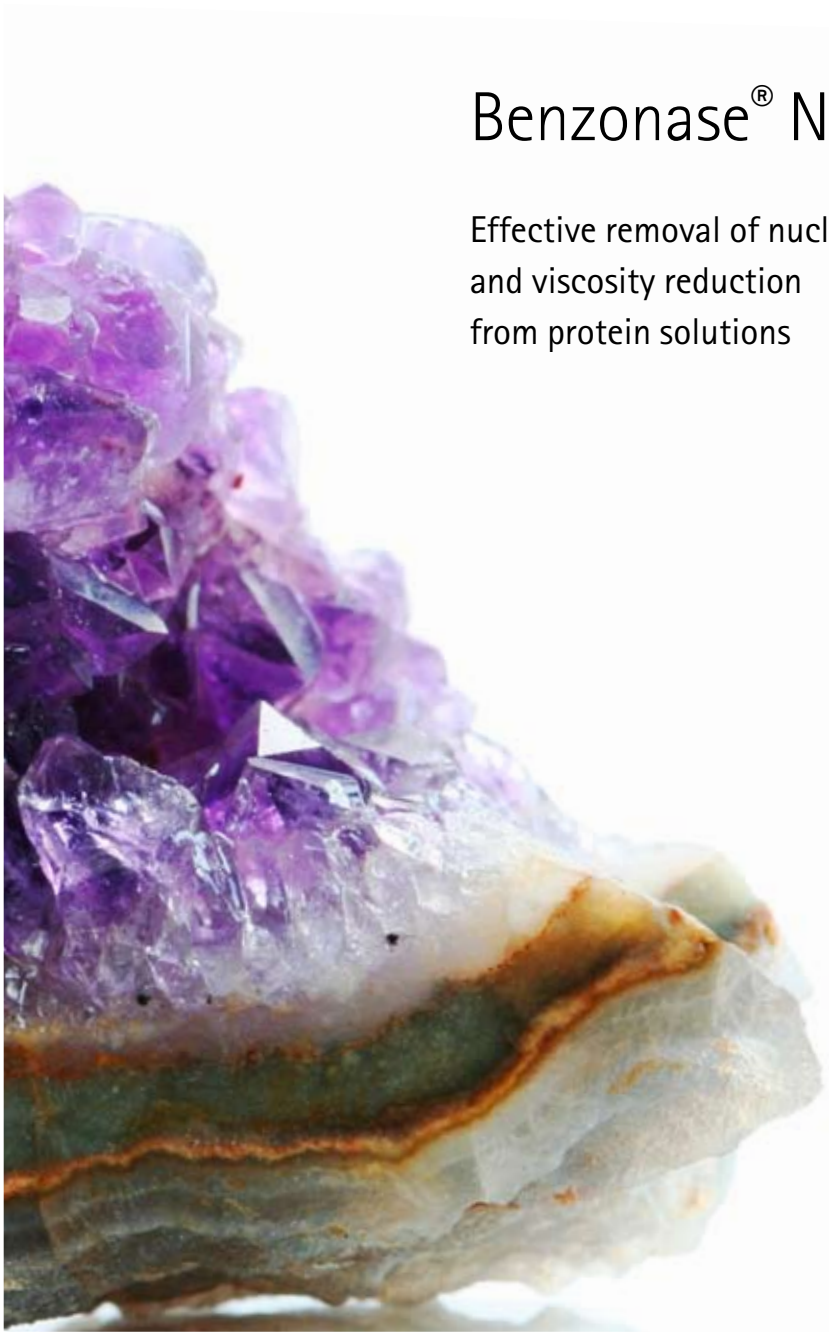

Benzonase® Nuclease

Effective removal of nucleic acids
and viscosity reduction
from protein solutions

EMD

Can I use Benzonase® Nuclease to make my protein experimental results perfectly clear?

Yes! By completely digesting nucleic acids, Benzonase® Nuclease reduces the viscosity of your samples – yet is perfectly safe for proteins. So you can enjoy sharper SDS-PAGE gels, better purifications, faster chromatography flow rates... and even a clearer mindset.

That's what's in it for you. EMD Chemicals

QUICK REFERENCE

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There is **only one effective biochemical method** to remove DNA and RNA. It is called **Benzonase® Nuclease**.

First commercialized by us over 15 years ago, Benzonase Nuclease is still manufactured only by **Merck KGaA Darmstadt, Germany**. Benzonase Nuclease has **proven its value** in the laboratory.

Why risk it with others...
....accept no substitute!

Benzonase® Nuclease

Degrades all forms of DNA and RNA ▶ *Works so well that there is no need for any other nuclease.*

Exceptionally high specific activity ▶ *You don't need to add a lot of extra 'protein' to your prep to get the results you want.*

Free of detectable proteolytic activity ▶ *For safe protein production processes and everyday applications.*

Effective over a wide range of operating conditions ▶ *Allows greater flexibility of reaction conditions to accommodate your protein needs.*

For current pricing on Benzonase® Nuclease or to access additional information, visit...

www.emdbiosciences.com/Benzonase

Benzonase® Nuclease is a genetically engineered endonuclease from *Serratia marcescens*. It degrades all forms of DNA and RNA (single-stranded, double-stranded, linear and circular) while having no proteolytic activity. The enzyme consists of two subunits of 30kDa each. It is effective over a wide range of conditions and possesses an exceptionally high specific activity. It completely digests nucleic acids to 5'-monophosphate terminated oligonucleotides 3 to 5 bases in length (below the hybridization limit), which is ideal for removal of nucleic acids from recombinant proteins enabling compliance with FDA guidelines for nucleic acid contamination.

Product	Size	Cat. No.
Benzonase® Nuclease, Purity >99%	10 KU	70664-3
Benzonase® Nuclease HC, Purity >99%	25 KU	71206-3
Benzonase® Nuclease, Purity >90%	2.5 KU 10 KU	70746-4 70746-3
Benzonase® Nuclease HC, Purity >90%	25 KU	71205-3

BULK

For Bulk and Custom options, please contact Technical Services:

Phone 800.628.8470
bioscienceshelp@emdchemicals.com

Ideal for a variety of applications including:

1 | Elimination of nucleic acid and viscosity from recombinant proteins

2 | Enhanced protein purification via complete cell lysis and viscosity reduction

3 | Increased gel resolution

4 | Prevention of cell clumping

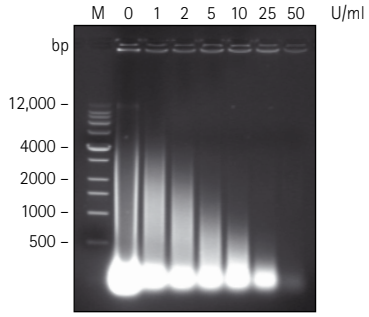
Applications

1 | *Elimination of nucleic acids and viscosity from recombinant proteins*

"Traditional" methods for the removal of interfering nucleic acids, such as extraction, sonication or precipitation, are not specific. These methods can be harsh and result in a loss in yield of protein due to denaturation and precipitation effects.

Many recombinant proteins have requirements concerning residual nucleic acids. This is a typical field of application for the use of Benzonase® Nuclease. In particular, Benzonase Nuclease completely digests nucleic acids (all kinds of DNA and RNA) to 5'-monophosphate-terminated oligonucleotides 3 to 5 bases in length. This is significantly below the hybridization limit, thus enabling recombinant proteins to meet the FDA guidelines for nucleic acid contamination.

Also, the ability of Benzonase Nuclease to rapidly hydrolyze nucleic acids makes the enzyme an ideal choice for reducing cell lysate viscosity consistently. This results in reduced processing times, increased protein yields, and increased efficiency of chromatographic purification steps.



Nucleic acid digestion by Benzonase

E. coli BL21(DE3) cells containing a pET construct were suspended in BugBuster Reagent® (5 ml/g wet weight). Samples of the suspension were treated with the indicated amounts of Benzonase for 30 min at room temperature. Samples were clarified by centrifugation and analyzed by agarose gel electrophoresis and ethidium bromide staining.

Destroy the nucleic acids, not your protein!

2 | *Enhanced protein purification via complete cell lysis and viscosity reduction*

When purifying proteins for functional or structural proteomics studies, the first step is to disrupt the cells or tissue samples and extract the relevant protein fraction. The purification process should be carried out without any time delay in order to minimize losses of the target protein by proteolytic attack.

However, cell extracts often show high viscosity caused by the release of nucleic acids during the disruption of cells. The addition of Benzonase® Nuclease rapidly hydrolyzes nucleic acids resulting in viscosity reduction thus reducing processing times and increasing protein yields.

For further purification enhancement and protein yield from *E. coli* cultures, the enzyme is compatible with BugBuster® and PopCulture® Protein Extraction Reagents enabling complete cell lysis, nucleic acid digestion and viscosity reduction all in the same step. This provides a simple, rapid, low-cost alternative to mechanical methods such as French press or sonication for releasing expressed target protein in preparation for purification.

Benzonase Nuclease can also be added to eukaryotic extracts obtained with CytoBuster™ and Insect PopCulture Protein Extraction Reagents to achieve the same protein purification enhancements seen with *E. coli* extracts.

Viscosity reduction by Benzonase Nuclease
E. coli BL21(DE3) cells containing a pET construct were suspended in BugBuster Reagent (5 ml/g wet weight). Aliquots of the suspension were treated with the indicated amounts of Benzonase Nuclease for 10 min at room temperature, centrifuged at 350 × g for 3 min and photographed.

Enhanced IMAC purification results using the combination of Benzonase® Nuclease and BugBuster® Protein Extraction Reagent

Media	Extraction	Flow rate ml/min	Recovery total mg	Media capacity mg/ml	Purity
His•Bind® Resin[1]	BugBuster only	0.7	32.1 mg	8.0	84%
His•Bind Resin[1]	BugBuster + Benzonase	7.5	37.1 mg	9.3	88%
Ni-NTA His•Bind Resin[2]	BugBuster only	0.5	31.6 mg	7.9	85%
Ni-NTA His•Bind Resin[2]	BugBuster + Benzonase	5.1	44.4 mg	7.9	90%

[1] *IDA Ni^{2+} chelation. Can be regenerated many times with no loss in performance. Note that chelating or other reducing agents can produce poor purification results due to higher metal leaching rates vs. NTA resins.*
[2] *NTA Ni^{2+} contain additional chelation site which minimizes leaching and is compatible with 20 mM β-mercaptoethanol for reduction of disulfide bonds.*

After expression of a recombinant protein in E. coli, the culture was split in half. One half of the cells were lysed using BugBuster® Protein Extraction Reagent and the other half was lysed with BugBuster plus Benzonase® Nuclease following the recommend protocol. Following the extraction, the extracts were equally split into two and the protein was purified using an either a His•Bind® Resin or a Ni-NTA His•Bind Resin column while measuring the flow rate of the columns. Subsequently, the yields and purities were determined.

For current pricing on Benzonase® Nuclease or to access additional information, visit...

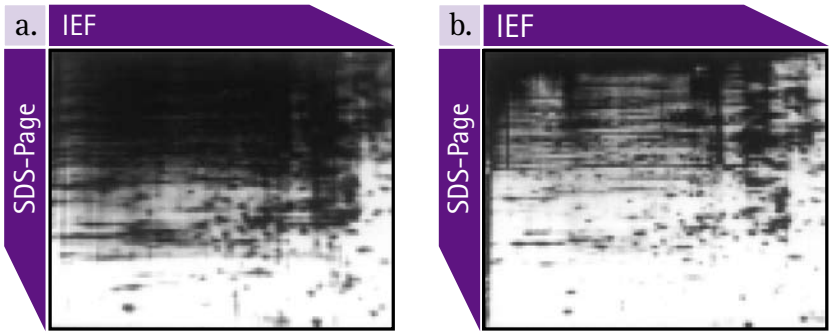
www.emdbiosciences.com/Benzonase

Bust and move your sample efficiently through purification!

Applications (continued)

3 | *Increased gel resolution*

Two-dimensional gel electrophoresis is a powerful technique in proteomics for the high resolution separation of complex mixtures of proteins. Nucleic acids are negatively charged molecules. They tend to form complexes by electrostatic interactions with positively charged domains on the surface of proteins. The formation and shape of these adducts usually cannot be predicted. These complexes behave completely different when migrating in an electric field than would be expected for a pure protein. Besides other effects, this may lead to band shifts in the typical protein patterns and cause poor resolution in two-dimensional gel electrophoresis.
Pre-treatment of the sample with Benzonase® Nuclease will significantly improve the resolution of electrophoretic separation as demonstrated in the figure below.



Silver-stained two dimensional gel electrophoretic spearation of bacterial cells (Proteus vulgaris)
a. without use of Benzonase Nuclease
b. by use of Benzonase Nuclease

Note: The presence of Benzonase Nuclease could not be detected on the gel.

4 | *Prevention of cell clumping*

A novel application of Benzonase® Nuclease is its incorporation into cell culture media to prevent cell clumping, especially when thawing frozen cell samples. Not only is Benzonase Nuclease free of protease activity, but it poses no threat to healthy cells making it ideal for such a role.

Recent studies demonstrate utility of Benzonase Nuclease in developing vaccines against some of the world's most serious infectious diseases. Peripheral Blood Mononuclear Cells (PBMCs) isolated from whole blood have important applications in vaccine research, for example, during quantification of vaccine-induced T-cell responses. T-cell response assay methods initially require freshly isolated cells for optimal signal detection, posing a serious practical limitation for sample handling during large clinical trials. However, frozen PBMCs (particularly PBMCs prepared from stored blood) tend to clump together upon thawing, preventing further analysis.

In 2001, Smith et al. (1) demonstrated that inclusion of Benzonase Nuclease in PBMC thawing buffer prevented cell clumping, allowing implementation of PBMC cryopreservation. This method has since been widely adopted in vaccine evaluation studies (2,3).

1. Smith, J.G., et al. 2001. *Clin. Diagn. Lab. Immunol.* **8**, 871.
2. Huaman, M.C., et al. 2008. *J. Immunol.* **180**, 1451.
3. Bull, M., et al. 2007. *J. Immunol. Meth.* **322**, 57

For current pricing on Benzonase® Nuclease or to access additional information, visit...

www.emdbiosciences.com/Benzonase

When your cells just want to be alone...

Enzyme Characteristics

We are able to meet the widest possible range of processing requirements because we are the only manufacturer of Benzonase® Nuclease. This enables us to offer the most options for your Benzonase Nuclease needs. Benzonase Nuclease is available in two different grades of purity and concentrations:

BULK

For Bulk and Custom options, please contact Technical Services:

Phone 800.628.8470
bioscienceshelp@emdchemicals.com

Enzyme	Purity	Concentration	Endotoxins levels	Specific activity	Comments
Benzonase Nuclease HC, Purity >99%	Ultra Pure	250 U/µl	<0.25 EU/1000 units	1 x 10^6 units/ mg protein	Produced by chromatographic purification; produces a single band by SDS-PAGE
Benzonase Nuclease, Purity >99%	Ultra Pure	25 U/µl	<0.25 EU/1000 units	1 x 10^6 units/ mg protein	
Benzonase Nuclease HC, Purity >90%	Pure	250 U/µl	Not tested	1 x 10^6 units/ mg protein	Produces the dominant band by SDS-PAGE
Benzonase Nuclease, Purity >90%	Pure	25 U/µl	Not tested	1 x 10^6 units/ mg protein	

Reaction Conditions

Benzonase Nuclease retains its activity in a wide range of reaction conditions, as specified in the following table and figures 1 to 3. The enzyme is also active in the presence of ionic and non-ionic detergents, reducing agents, PMSF (1 mM), EDTA (1 mM) and urea (relative activity depends on specific conditions). Activity is inhibited by >300 mM monovalent cations, >100 mM phosphate, >100 mM ammonium sulfate, or >100 mM guanidine HCl.

Condition	Optimal*	Effective**
Mg^{2+} concentration	1 – 2 mM	1 – 10 mM
pH	8.0 - 9.2	6.0 – 10.0
Temperature	37 °C	0 – 42 °C
Dithiothreitol (DTT)	0 – 100 mM	> 100 mM
ß-Mercaptoethanol	0 – 100 mM	> 100 mM
Monovalent cation concentration (Na^+, K^+, etc.)	0 – 20 mM	0 – 150 mM
PO_4^{3-} concentration	0 – 10 mM	0 – 100 mM

** "Optimal" is defined as the condition in which Benzonase Nuclease retains ≥90 % of its activity.*
*** "Effective" is defined as the condition in which Benzonase Nuclease >15 % of its activity.*

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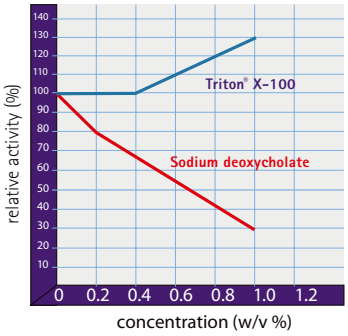
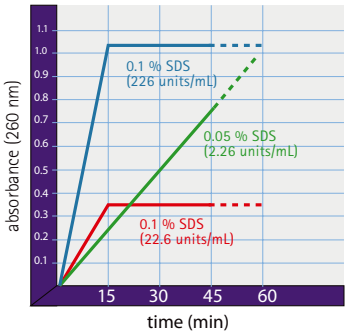
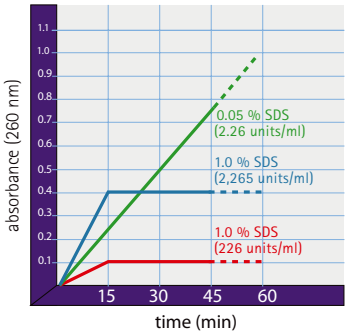
Effect of detergents (Triton® X-100, sodium deoxycholate) on Benzonase® Nuclease activity*



- Concentrations of Triton® X-100 < 0.4% have no effect on the activity of Benzonase Nuclease.
- At concentrations of sodium deoxycholate < 0.4%, Benzonase Nuclease retains at least 70% of its activity.

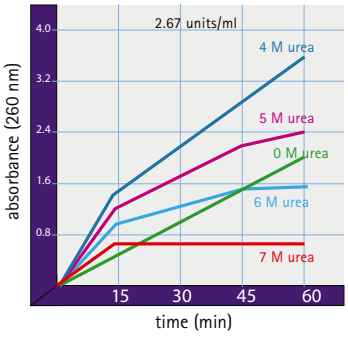
Effect of different SDS concentrations on Benzonase Nuclease activity†

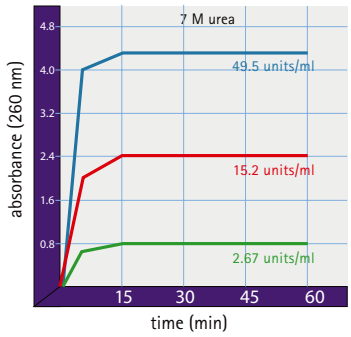




- Benzonase Nuclease retains 100% of its activity in SDS concentrations up to 0.05%.
- At SDS concentrations between 0.1% and 1%, Benzonase Nuclease remains active for a short period of time before being denatured. This can be partially compensated by increasing the concentration of Benzonase Nuclease.

Effect of different concentrations of urea on Benzonase Nuclease activity*†





- Benzonase Nuclease is activated by urea of concentrations up to approximately 6M.
- At 6M urea, enzyme activity first increases, then decreases over time.
- At 7M urea, Benzonase Nuclease denatures after 15 min, and activity is lost. However, significant degradation of nucleic acids occurs before the enzyme is inactivated.
- Higher concentrations of Benzonase Nuclease can partially compensate the effects of 7M urea.

* Standard assay conditions = 100% activity. Substrate is DNA.
†Benzonase Nuclease concentrations are given in units/ml.

Reaction conditions defined by your needs, not your enzymes.

Kari Weber, Ph.D.
EMD Chemicals – Novagen

Frequently Asked Questions

What you need to know about Benzonase® Nuclease

Which quality of Benzonase Nuclease will be adequate for certain applications? What does HC mean?

There are several parameters that influence the activity of Benzonase Nuclease, such as the incubation time, incubation temperature, and the quantity used. Hence, the optimal purity/amount of the nuclease and the optimal conditions will vary from process to process and needs to be determined experimentally. For viscosity reduction, Benzonase Nuclease, Purity >90% will often be sufficient, especially if *E. coli* is used as the expression system. In addition to the standard concentration of 25 U/µl, Benzonase Nuclease is also provided in a High Concentration (HC) formulation of 250 U/µl.

What type of nucleic acids does Benzonase Nuclease work on? Can I use it when isolating RNA? What is the end result of the digestion?

Benzonase Nuclease is a promiscuous endonuclease that attacks and degrades all forms of DNA and RNA (single stranded, double stranded, linear, circular, and super-coiled). Upon complete digestion, all nucleic acids present in the solution are reduced to 5'-monophosphate-terminated oligonucleotides 3 to 5 bases in length.

For what purposes would I want to use Benzonase Nuclease?

Some applications include: elimination of nucleic acids, reduction of viscosity to facilitate processing (e.g., recombinant protein purification, viscosity-sensitive applications using mammalian cell lysates, etc.); reduction of cell clumping in stored peripheral blood mononuclear cell (PBMC) samples prior to processing (see Perrin, 2008 *inNovations* 28, 21); preparation of inclusion bodies to allow successful protein renaturation; and removal of negatively charged nucleic acids from samples prior to two-dimensional SDS-PAGE.

How can Benzonase Nuclease be inactivated? How can it be removed?

Reversible inhibition can be achieved using EDTA to chelate essential metal ions. Irreversible inactivation can only be accomplished with extreme conditions (100 mM NaOH at 70°C for 30 min). The enzyme can easily be separated from the target product using chromatography. Please note that Benzonase Nuclease consists of two subunits of 30 kDa each. Because of the robust nature of this endonuclease, we recommend that Benzonase Nuclease not be used if a nuclease-free end product is required. For more information, visit www.novagen.com/benzonase.

My Benzonase Nuclease was left out on the bench all weekend. Is it still good?

We have done extensive stability testing on Benzonase Nuclease, and find that it is extremely stable. Even with extended incubations at 37°C, Benzonase Nuclease maintained >90% activity for several months. For more information on stability testing, go to www.novagen.com/benzonase.

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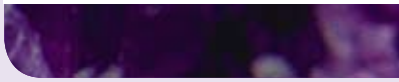


I want to use a different buffer. What conditions are absolutely required for full activity of Benzonase® Nuclease? What will reduce its activity?

Benzonase Nuclease requires 1-2 mM Mg^{2+} for activity. It is inhibited (approximately 50% activity) by monovalent cation concentrations >300 mM, phosphate concentrations >100 mM, and by ammonium sulfate concentrations >100 mM. In addition, concentrations of >1mM EDTA will also inhibit Benzonase Nuclease activity.

Is Benzonase Nuclease compatible with protease inhibitor cocktails?

Benzonase Nuclease is compatible with protease inhibitors. However, caution should be exercised, since many protease inhibitor cocktails include EDTA. As mentioned previously, concentrations of >1 mM EDTA will inhibit Benzonase Nuclease activity.

My protein is insoluble and I need to perform purification under denaturing conditions. Will Benzonase Nuclease still work in urea?

Benzonase Nuclease activity actually increases in presence of urea at concentrations up to 6 M (see page 9). At 6 M urea, enzyme activity first increases, then decreases over time. At 7 M urea, Benzonase Nuclease denatures after 15 min, and activity is lost. However, significant degradation of nucleic acids occurs before inactivation. Higher initial concentrations of Benzonase Nuclease can partially compensate the effects of 7 M urea.

How much Benzonase Nuclease should I use?

One unit of Benzonase Nuclease is defined as the amount of enzyme that results in an ΔA_{260} of 1.0 in 30 min, which corresponds to complete digestion of 37 µg DNA. When used in conjunction with BugBuster® or CytoBuster™ Protein Extraction Reagents, we recommend the addition of 25 U Benzonase Nuclease per 1 ml cell lysate (assuming BugBuster or CytoBuster reagents are used at standard concentrations). Conditions may need to be altered when working at lower temperature, as is the case with most mammalian cell lysis protocols. For example, when using the Benzonase Nuclease at 4°C, we recommend increasing the duration of the digestion.

Related Products

Being the gold standard in protein research, we also offer many other valuable tools to accommodate your protein purification needs. To propel your research forward, don't forget to ask your local technical sales specialist about how else we can help!

BugBuster® Master Mix

Product	Size	Cat. No.
BugBuster® Master Mix	100 ml	71456-3
	500 ml	71456-3

BugBuster Master Mix combines BugBuster Protein Extraction Reagent with Benzonase® Nuclease and rLysozyme™ Solution into one convenient reagent. BugBuster® Protein Extraction Reagent is formulated to gently disrupt the cell wall of *E. coli* and liberate soluble proteins. It provides a simple, rapid, low-cost alternative to mechanical methods such as French press or sonication for releasing expressed target protein in preparation for purification or other applications. The proprietary formulation utilizes a detergent mix that is capable of cell wall perforation without denaturing soluble protein. BugBuster Master Mix allows for maximum recovery of active, soluble protein from both Gram-negative and Gram-positive bacteria. BugBuster Master Mix combines the activities of BugBuster Reagent, rLysozyme solution and Benzonase Nuclease, thus significantly increasing protein extraction efficiency and facilitating downstream processing of protein extracts. The two available package sizes provide sufficient reagents for protein extraction from 20 g and 100 g cell paste.

Lysonase™ Bioprocessing Reagent

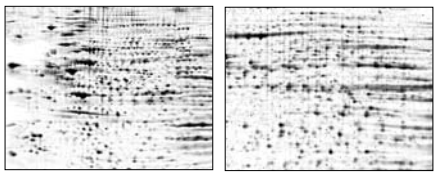
Product	Size	Cat. No.
Lysonase™ Bioprocessing Reagent	0.2 ml	71230-3
	1 ml	71230-4
	5 x 1 ml	71230-5

Lysonase™ Bioprocessing Reagent is an optimized, ready-to-use blend of rLysozyme Solution and Benzonase Nuclease. rLysozyme Solution contains a highly purified and stabilized recombinant lysozyme with specific activity 250 times greater than that of chicken egg white lysozyme. The combined activities of rLysozyme and Benzonase Nuclease significantly increase protein extraction efficiency and facilitate downstream processing of protein extracts. For efficient protein extraction with BugBuster Protein Extraction Reagent, use 10 µl Lysonase per 1 g cell paste. In addition, Lysonase can be used to enhance the effectiveness of non-detergent based cell lysis procedures.

ProteoExtract® Complete Protein Extraction Kit

Product	Size	Cat. No.
ProteoExtract® Complete Mammalian Proteome Extraction Kit	1 kit	539779

The ProteoExtract® Complete Proteome Extraction Kit (C-PEK) is designed for fast and easy extraction of total proteins from mammalian cells and tissues, without the need for sonication or precipitation. C-PEK provides a straightforward two-step isolation of complete proteomes in a single microcentrifuge tube. It uses optimized extraction reagents to provide improved protein solubilization, resulting in an increased number of resolved spots on 2D gels. The procedure uses proprietary Benzonase Nuclease, leading to clear, nonviscous protein solutions and improved resolution on 2D gels. Using the C-PEK procedure, protein concentration is not necessary. Extracted proteins are ready for immediate use in standard downstream proteomics applications. Each kit contains all the reagents needed for 20 reactions ($1\text{-}2 \times 10^8$ cells per reaction).



Different samples show high resolution in 2D gel electrophoresis.

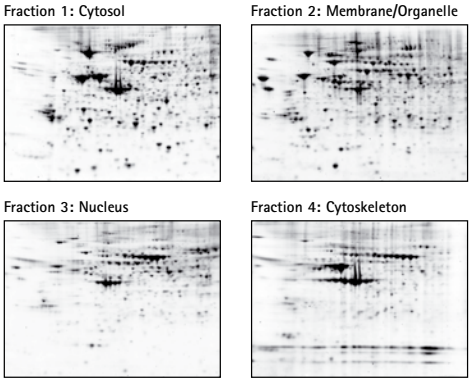
For current pricing on Benzonase® Nuclease or to access additional information, visit...

www.emdbiosciences.com/Benzonase

ProteoExtract® Subcellular Protein Extraction Kit

ProteoExtract Subcellular Proteome Extraction Kit (S-PEK) is designed for fast and reproducible extraction of subcellular proteomes from adherent and suspension-grown mammalian cells. The S-PEK takes advantage of the different solubilities of certain subcellular compartments in the four selected reagents. The extraction procedure utilizes four extraction buffers prepared with ultra-pure chemicals, a protease inhibitor cocktail to prevent protein degradation during the extraction procedure, and Benzonase® Nuclease to degrade contaminating nucleic acids. In the case of adherent cells, the procedure is performed directly in the tissue culture dish without the need for cell removal. Cells or the parts of the cells remain attached to the plate during sequential extraction of subcellular compartments, until the appropriate extraction reagent is used. Thus, the early destruction of the cellular structure by enzymatic or mechanical detachment of cells from the tissue culture plate and any mixing of different subcellular compartments is prevented. For suspension-grown cells, extraction starts with gentle sedimentation and washing of the cells. For tissues, fragmentation is required before proceeding with the extraction protocol. The stepwise extraction delivers four distinct protein fractions from one sample: cytosolic fraction; nucleic protein fraction; membrane/organelle protein fraction; and cytoskeletal fraction.

Proteins are obtained in the native state (except the cytoskeletal fraction), making the S-PEK suitable for many downstream applications such as 1D and 2D gel electrophoresis, immunoblotting, enzyme activity assays, and protein microarrays. Each kit contains all reagents needed for 20 reactions (Cat. No. 539790) or 10 reactions (Cat. No. 539791). Sample size: 1×10^6 or 25-50 mg tissue.



Protein profiling by 2D gel electrophoresis

The comparison of fractions 1 through 4 by two-dimensional SDS-PAGE indicates a large number of protein spots that are specific to each respective subcellular fraction.

Product	Size	Cat. No.
ProteoExtract® Subcellular Proteome Extraction Kit	1 kit	539790
ProteoExtract® Subcellular Proteome Extraction Kit, Mini	1 kit	539791

USER CITATIONS

As discussed earlier, Benzonase® Nuclease is ideal for a variety of applications including reduction of viscosity caused by nucleic acids, purification of proteins, sample preparation for electrophoresis and chromatography and prevention of cell clumping in the cell culture media. Below are selected citations that demonstrate the use of Benzonase Nuclease for each of these applications.

Elimination of Nucleic Acids and Viscosity from Recombinant Proteins

Shi, H., et. al. (2009) Mitogen-activated protein kinase signaling is necessary for the maintanence of skeletal muscle mass. *Am. J. Physiol. Cell Physiol.* **296**, C1040.

Hervas-Stubbs , S., et al. (2007) Insect baculoviruses strongly potentiate adaptive immune responses by inducing type I IFN. *J. Immuno.* **178**, 2361.

Kercher, L., et al. (2007) Prion protein (PrP) expression differences in microglia and astroglia influence scrapie-induced neurodegeneration in retina and brain of transgenic mice. *J. Virology* **81**, 10340.

Cho, S., et al. (2005) Glucocorticoid receptor ligand binding domain is sufficient for the modulation of glucocorticoid induction properties by homologous receptors, coactivator transcription intermediary factor 2, and Ubc9. *Mol. Endocrinology* **19**, 290.

Han, W., et al. (2005) C-terminal ECFP fusion impairs synaptotagmin 1 function: crowding out synaptotagmin 1. *J. Biol. Chem.* **280**, 5089-5100.

Meyn, M., et al. (2005) Src family kinase activity is required for murine embryonic stem cell growth and differentiation. *Mol. Pharmacol.* **68**, 1320.

Murphy, M., et al. (2003) An improved method for the *in vitro* evolution of aptamers and applications in protein detection and purification. *Nucleic Acids Res.* **31**, e110.

Persad, S., et al. (2001) Regulation of protein kinase B/Akt-serine 473 phosphorylation by integrin-linked kinase. *J. Bio. Chem.* **276**, 27462.

Enhanced Protein Purification via Complete Cell Lysis and Viscosity Reduction

Collin, R., et. al. (2009) The DEXD/H-box RNA Helicase DDX19 is regulated by an α-helical switch. *J. Biol. Chem.* **284**, 10296.

Gustin, K., et. al. (2009) Bovine coronavirus nsp1 (p28) is an RNA binding protein that binds terminal genomic cis-replication elements. *J. Viol.* **10.1128/JVI**, 00160.

Wanyiri, J., et. al. (2009) Role CpSUBI, a subtillisin-like protease, in *Cryptosporidium parvum* infection *in vitro*. *Eukaryot. Cell.* **8**, 470.

Shen, X., et. al. (2009) Identification of novel SHPS-1-associated proteins and their roles in regulation of insulin-like growth factors-dependent responses in vascular smooth muscle cells. *Mol. Cell. Proteomics.* **10.1074/MCP**, M800543.

Heikkilä, O., et. al. (2009) Integrin αVβ6 is a high-affinity receptor for coxsackievirus A9. *J. Gen. Virol.* **90**, 197.

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