BugBuster® Protein Extraction Reagent

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About the Kits

BugBuster® Protein Extraction Reagent	100 ml	70584-3
	500 ml	70584-4
BugBuster Plus Benzonase® Nuclease	1 kit	70750-3
BugBuster Plus Lysonase™ Kit	1 kit	71370-3
	1 kit	71370-4
BugBuster 10X Protein Extraction Reagent	10 ml	70921-3
	50 ml	70921-4
BugBuster (primary amine-free) Extraction Reagent	100 ml	70923-3
	500 ml	70923-4
BugBuster HT Protein Extraction Reagent	100 ml	70922-3
	500 ml	70922-4
BugBuster Master Mix	100 ml	71456-3
	500 ml	71456-4

Description

BugBuster® Protein Extraction Reagent is formulated for gentle disruption of *E. coli* cell wall to liberate active 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 Tris-buffer based mixture of non-ionic and zwitterionic detergents that is capable of cell wall perforation without denaturing protein.

In practice, cells are harvested by centrifugation and suspended in BugBuster reagent. At this point, Benzonase® Nuclease can be added to reduce viscosity of extract caused by liberation of chromosomal DNA. The addition of highly specific rLysozyme™ Solution, which hydrolyzes *N*-acetylmuramide linkages in the peptidoglycan layer of the cell wall enhances the extraction efficiency, especially for larger proteins. rLysozyme is unnecessary when extracting protein from host strains harboring the pLysS plasmid as they express T7 lysozyme, e.g., BL21 (DE3) pLysS. Following a brief incubation, insoluble cell debris is removed by centrifugation. The clarified extract is ready to use and fully compatible with affinity supports offered by Novagen. Following binding to affinity resin, excess BugBuster is easily removed by washing the column with appropriate buffer.

BugBuster is also useful for the preparation of high-purity inclusion bodies for subsequent purification in instances where expressed proteins are insoluble.

BugBuster Protein Extraction Reagent

The standard BugBuster reagent is supplied as a Tris-buffered "1X" ready-to-use liquid that is stable at room temperature. The two BugBuster sizes provide sufficient reagents for protein extraction from either 20 or 100 g cell paste.

BugBuster Plus Benzonase Nuclease

The 500-ml size of the BugBuster reagent is also available bundled with 10,000 U Benzonase Nuclease (provided in a separate vial) for the preparation of low viscosity extracts and/or removal of nucleic acids from protein preparations.

BugBuster Plus Lysonase Kit

Lysonase Bioprocessing reagent is an optimized, ready-to-use blend of rLysozyme Solution and Benzonase Nuclease. BugBuster Plus Lysonase Kit combines the features of both reagents to significantly increase protein extraction efficiency and facilitate downstream processing of protein extracts, thereby enabling maximum recovery of active soluble protein. The two BugBuster Plus Lysonase Kit sizes provide sufficient reagents for protein extraction from either 20 or 100 g cell paste.

BugBuster® 10X Protein Extraction Reagent

BugBuster® 10X is a concentrated formulation of the proprietary detergents employed in BugBuster without buffer components, allowing user-defined dilution. BugBuster 10X has the same bioprocessing benefits as standard BugBuster plus the freedom to control pH, reagent concentration, and buffer additives necessary for maximum extraction and activity of target protein.

BugBuster (primary amine-free) Extraction Reagent

BugBuster (primary amine-free) is a unique formulation of BugBuster designed for applications where primary amines would interfere if present in protein extracts, such as protein immobilization or cross-linking. The PIPPS buffer used in BugBuster (primary amine-free) has a similar buffer capacity and pH range as the original Tris-buffered BugBuster, but will not complex metal ions, also making it ideally suited for extraction of metal-dependent proteins.

BugBuster HT Protein Extraction Reagent

BugBuster HT combines BugBuster Protein Extraction Reagent and Benzonase® Nuclease in one convenient reagent. The resulting protein extract can easily be fractionated by conventional purification techniques. BugBuster HT is ideally suited for application in high-throughput protein purifications.

BugBuster Master Mix

BugBuster Master Mix is an all-in-one reagent that combines BugBuster Protein extraction Reagent with Benzonase Nuclease and rLysozyme™ Solution in one convenient formulation. BugBuster Master Mix allows for maximum recovery of active soluble protein. With the Master Mix, there is no need for dilution or separate addition steps. The two available sizes provide sufficient reagents for protein extraction from 20g and 100 g cell paste.

Components

BugBuster Plus Benzonase Nuclease

- 500 ml BugBuster Protein Extraction Reagent
- 10 KU Benzonase Nuclease

BugBuster Plus Lysonase Kit

- 100 or 500 ml BugBuster Protein Extraction Reagent
- 0.2 or 1 ml Lysonase™ Bioprocessing Reagent

All others

See p 2.

Storage

Store BugBuster, BugBuster (primary amine-free), and BugBuster 10X Protein Extraction Reagent at room temperature. Store BugBuster HT and BugBuster Master Mix at 4°C.

Benzonase Nuclease is supplied in 50% glycerol containing 50 mM Tris-HCl, 20 mM NaCl, and 2 mM $MgCl_2$, pH 8.0. The enzyme preparation is stable for one year when stored at –20°C. **DO NOT store at –70°C because freezing Benzonase Nuclease results in loss of activity.**

Store Lysonase Bioprocessing Reagent at –20°C. **DO NOT store at –70°C because freezing Lysonase results in loss of activity.**

Note: Storage of BugBuster HT and BugBuster Master Mix at temperatures at 4°C may cause precipitation of detergents. Before use, redissolve the detergent precipitates by incubating in a room temperature water bath with gentle swirling. Once redissolved, the BugBuster formulations maintain their extraction efficiency.

Considerations before you begin

- BugBuster® can be used on fresh or frozen cell pellets. For comparisons of multiple samples (e.g., extended time course analysis), all cell pellets should be processed identically (all fresh or all frozen). Superior extraction efficiencies can be obtained by freezing the cell pellet before resuspending in BugBuster. However, some target proteins may be inactivated by freeze-thaw cycles.
- For optimal extraction, especially of high molecular weight proteins (> 70 kDa), addition of rLysozyme™ Solution (Cat. No 71110-3, for non-pLysS or pLysE hosts) and/or freezing of bacterial cell pellets prior to extraction with BugBuster reagent is highly recommended.
- BugBuster Protein Extraction Reagent and Benzonase® Nuclease are most efficient when used at room temperature. Storage of BugBuster at 4°C or below may cause precipitation of the detergents. Prior to use, redissolve any precipitants by incubation in a room temperature water bath with gentle swirling.
- Extraction efficiency can be strain-dependent and appears to be especially efficient with the BL21 strain and its derivatives. The use of pLysS or pLysE hosts also enhances extraction due to the activity of T7 lysozyme.
- BugBuster is compatible with Tris and phosphate-based buffer systems in the near-neutral pH range. The detergents in BugBuster will precipitate at or above 1 M NaCl. Evaluate extraction on a small scale when using high salt buffers or acid or alkaline pH ranges for chromatography. BugBuster is fully compatible with all Novagen chromatography resins.
- The following guidelines should be considered before diluting BugBuster 10X Protein Extraction Reagent for use.
    - Certain components of BugBuster are unstable at acidic pH. Avoid diluting BugBuster 10X in buffers below pH 5.0. BugBuster can safely be diluted in basic buffer systems (pH 8.0–10.0).
    - BugBuster is compatible with salt concentrations of up to 1.0 M. At higher salt concentrations, BugBuster may precipitate, depending on the pH and temperature.
    - BugBuster is compatible with phosphate, Tris, and PIPPS buffer systems. Test a small volume of BugBuster 10X by dilution in other buffer systems to confirm that precipitation will not occur.
- BugBuster reagent is compatible with EDTA, however EDTA will interfere with protein binding to metal affinity resins such as our His•Bind® and Ni-NTA His-Bind Resins.
- BugBuster reagent is compatible with protease inhibitors. We recommend Protease Inhibitor Cocktail Set II (with EDTA) (Cat. No. 539132) or Protease Inhibitor Cocktail Set III (without EDTA) (Cat. No. 539134) with *E. coli* extracts. The EDTA-free cocktail (Cat. No. 539134) is recommended for extracts that are going to be subsequently used for metal affinity purification, e.g., with our His-bind(R) resins. However, serine protease inhibitors should be avoided if the target protein is to be treated with Thrombin (Cat. No. 69671-3), Factor Xa (Cat. No. 69036-3) or Recombinant Enterokinase (Cat. No. 69066-3). Although purification may remove active inhibitors, dialysis or gel filtration is recommended prior to cleavage.
- BugBuster reagent is compatible with reducing agents such as Tris(hydroxypropyl)phosphine (THP), β-mercaptoethanol, and DTT. Note that reducing agents may activate proteases. If the target protein requires a reducing environment, 0.5 M THP Solution (Cat. No. 71194) is recommended. THP is a water-soluble, odorless, ready to use, neutral reducing agent that is more stable and effective than DTT as a sulfhydryl reductant. THP is more resistant to air oxidation than DTT and is compatible at a concentration of 1.0 mM for use in immobilized metal affinity chromatography with His•Bind® and Ni-NTA His•Bind Resins. Up to 20 mM β-mercaptoethanol can be used with Ni-NTA His•Bind Resin.
- When processing small quantities of cell paste with BugBuster and Benzonase, Benzonase Nuclease can be diluted with 50 mM Tris-HCl, 20 mM NaCl, 2 mM $MgCl_2$, pH 8.0. This solution can be stored at 4°C for several days without loss of activity.
- **Benzonase Nuclease is not recommended for nuclease-free preparations.** Depending on the protein purification method, Benzonase may be removed during processing. Residual nuclease activity can be monitored by incubation of the purified protein with RNA or DNA markers followed by gel analysis.
- Benzonase activity is inhibited by approximately 50% when monovalent cation concentrations > 50 mM, phosphate concentrations > 20 mM, and ammonium sulfate concentrations > 25 mM. are used.

- BugBuster®, Benzonase®, and rLysozyme™ are compatible with the BCA Protein Assay (Cat. No. 71285-3), GST•Tag™ Assay (Cat. No. 70532-3), S•Tag™ Rapid Assay (Cat. No. 69212-3), or FRETWorks™ S•Tag Assay (Cat. No. 70724-3). Dilute the standards in the same reagent(s) used for extract preparation.
- For SDS-PAGE and Western blot analysis of BugBuster extracts prepared by the recommended protocols, generally an extract load volume of approximately 2–5 µl well (in a 15-well mini gel) would be sufficient to visualize the different protein bands in the soluble protein fraction. This amount should be used as a guideline only as the optimal amount of material to load will vary with the expression level of the target protein, the efficiency of the extraction, detection sensitivity of the Western blot method, etc.
- BugBuster in conjunction with Lysonase bioprocessing reagent has been used for successfully extracting protein from certain gram-positive species such as *B. subtilis* and *E. faecalis*. Due to the thicker peptidoglycan layer in the cell walls of gram-positive bacteria, extraction will be more efficient if more Lysonase than recommended for *E. coli* is used. For efficient protein extraction with gram-positive bacteria, use 5 ml of BugBuster and 40 µl of Lysonase per gram wet cell paste followed by a 20-minute incubation at room temperature.

Soluble Fraction

The soluble fraction prepared using BugBuster will consist of soluble proteins from the periplasm and cytoplasm. If a separate periplasmic fraction is desired, follow the osmotic shock procedure in User Protocol TB055 or other suitable method. The pellet prepared from that procedure can be used in this protocol.

BugBuster Protein Extraction Reagent

The following protocol is for obtaining the soluble fraction when using BugBuster Protein Extraction Reagent, BugBuster 10X Protein Extraction Reagent, BugBuster (primary amine-free) Protein Extraction Reagent, BugBuster Plus Benzonase Nuclease and BugBuster Plus Lysonase Kit.

1. Harvest cells from liquid culture by centrifugation at 10,000 × g for 10 min using a weighed centrifuge tube. For small scale extractions (1.5 ml or less), centrifugation can be performed in a 1.5-ml tube at 14,000–16,000 × g. Decant and allow the pellet to drain, removing as much liquid as possible. Determine the wet weight of the pellet.

Note: *If using BugBuster 10X Protein Extraction Reagent, dilute to 1X in desired buffer.*

2. Resuspend the cell pellet in room temperature BugBuster reagent by pipetting or gentle vortexing, using 5 ml reagent per gram of wet cell paste. This typically corresponds to about 2.5 ml per 50-ml culture. For small cultures, use up to 1/5 culture volume for resuspension (e.g., use 300 µl BugBuster for 1.5-ml cultures). There are no adverse effects to using larger volumes of BugBuster, if needed.
3. **Optional but highly recommended:** To reduce viscosity of the lysate, add 1 µl (25 units) Benzonase® Nuclease per 1 ml BugBuster reagent (125 Units/gram cell paste) used for resuspension. Although Benzonase requires Mg^{2+} for activation, no addition of Mg^{2+} is required for viscosity reduction or nucleic acid digestion under the conditions described here.
4. **Optional but highly recommended:** To improve protein extraction efficiency in non-pLysS and -pLysE hosts, add 1 KU rLysozyme™ Solution per 1 ml BugBuster reagent (5 KU/gram cell paste). rLysoyzme Solution can be diluted using rLysozyme Dilution Buffer (100 mM NaCl, 50 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton® X-100, pH 7.5). Dilutions should be stored on ice or at 4°C, but used as soon as possible after dilution.

Note: *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.*

5. **Optional:** Add protease inhibitors. Protease inhibitors are compatible with BugBuster, Benzonase and rLysozyme. Please see the *General considerations* section on p 4 for recommended protease inhibitors.
6. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature.

Note: *If Benzonase was added, the extract should not be viscous at the end of the incubation.*

7. Remove insoluble cell debris by centrifugation at 16,000 × g for 20 min at 4°C. If desired, save the pellet for inclusion body purification as described on p 7.
8. Transfer the supernatant to a fresh tube. For SDS-PAGE analysis, remove a small sample (25–50 µl) and combine with equal volume of 4X SDS Sample Buffer (Cat. No. 70607-3). Immediately heat for 3 min at 85°C to denature proteins and then store at –20°C until SDS-PAGE analysis.
9. The remaining soluble extract can be loaded directly onto any Novagen purification resin (and numerous other systems). Maintain clarified extracts on ice for short term storage (2–3 h) or freeze at –20°C until needed. Extracts should be stored at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.

BugBuster® HT Protein Extraction Reagent

The following protocol is for obtaining the soluble fraction when using BugBuster® HT Protein Extraction Reagent.

1. Harvest cells from liquid culture by centrifugation at 10,000 × g for 10 min using a weighed centrifuge tube. For small scale extractions (1.5 ml or less), centrifugation can be performed in a 1.5-ml tube at 14,000–16,000 × g. Decant and allow the pellet to drain, removing as much liquid as possible. Determine the wet weight of the pellet.
2. Resuspend the cell pellet in room temperature BugBuster HT reagent by pipetting or gentle vortexing, using 5 ml reagent per gram of wet cell paste. This typically corresponds to about 2.5 ml per 50-ml culture. For small cultures use up to 1/5 culture volume for resuspension (e.g., use 300 µl BugBuster HT for 1.5-ml cultures). There are no adverse effects to using larger volumes of BugBuster HT, if needed.
3. **Optional but highly recommended:** To improve protein extraction efficiency in non-pLysS and -pLysE hosts, add 1 KU rLysozyme™ Solution per 1 ml BugBuster HT reagent (5 KU/gram cell paste). rLysoyzme Solution can be diluted using rLysozyme Dilution Buffer (100 mM NaCl, 50 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton® X-100, pH 7.5). Dilutions should be stored on ice or at 4°C, but used as soon as possible after dilution.

Note: rLysozyme can be pre-mixed with BugBuster HT for rapid sample processing. Prepare the rLysozyme and BugBuster HT mixture immediately before use.

4. **Optional:** Add protease inhibitors. Protease inhibitors are compatible with BugBuster HT and rLysozyme. Please see the *General considerations* section on p 4 for recommended protease inhibitors.
5. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature.

Note: The extract should not be viscous after incubation.

6. Remove insoluble cell debris by centrifugation at 16,000 × g for 20 min at 4°C. If desired, save the pellet for inclusion body purification as described on p 7.
7. Transfer the supernatant to a fresh tube. For SDS-PAGE analysis, remove a small sample (25–50 µl) and combine with equal volume of 4X SDS Sample Buffer (Cat. No. 70607-3). Immediately heat for 3 min at 85°C to denature proteins and then store at –20°C until SDS-PAGE analysis.
8. The remaining soluble extract can be loaded directly onto any Novagen purification resin (and numerous other systems). Maintain clarified extracts on ice for short term storage (2–3 h) or freeze at –20°C until needed. Extracts should be stored at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.

BugBuster Master Mix

The following protocol is for obtaining the soluble fraction when using BugBuster Master Mix.

1. Harvest cells from liquid culture by centrifugation at 10,000 × g for 10 min using a weighed centrifuge tube. For small scale extractions (1.5 ml or less), centrifugation can be performed in a 1.5-ml tube at 14,000–16,000 × g. Decant and allow the pellet to drain, removing as much liquid as possible. Determine the wet weight of the pellet.
2. Resuspend the cell pellet in room temperature BugBuster Master Mix by pipetting or gentle vortexing, using 5 ml reagent per gram of wet cell paste. This typically corresponds to about 2.5 ml per 50-ml

culture. For small cultures use up to 1/5 culture volume for resuspension (e.g., use 300 µl BugBuster® Master Mix for 1.5 ml cultures). There are no adverse effects to using larger volumes of BugBuster Master Mix.

3. **Optional:** Add protease inhibitors. Protease inhibitors are compatible with BugBuster Master Mix. Please see the *General considerations* section on p 4 for recommended protease inhibitors.
4. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature.

Note: The extract should not be viscous after incubation.

5. Remove insoluble cell debris by centrifugation at 16,000 × g for 20 min at 4°C. If desired, save the pellet for inclusion body purification as described on p 7.
6. Transfer the supernatant to a fresh tube. For SDS-PAGE analysis, remove a small sample (25–50 µl) and combine with equal volume of 4X SDS Sample Buffer (Cat. No. 70607-3). Immediately heat for 3 min at 85°C to denature proteins and then store at –20°C until SDS-PAGE analysis.
7. The remaining soluble extract can be loaded directly onto any Novagen purification resin (and numerous other systems). Maintain clarified extracts on ice for short term storage (2–3 h) or freeze at –20°C until needed. Extracts should be stored at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.

Inclusion Body Preparation

The following protocol can be used for inclusion body (IB) purification when using any of the BugBuster formulations.

1. To separate the soluble protein fraction from your sample, process the induced culture according to steps mentioned in the *Soluble Fraction* section on p 5–7. Save the pellet obtained after incubation of the cell suspension with BugBuster reagent and subsequent centrifugation. The supernatant obtained after the centrifugation contains the soluble fraction and can be saved for analyzing and purifying proteins that might partition in both the soluble and insoluble protein fraction.
2. Resuspend the pellet obtained after centrifugation in the same volume of BugBuster Protein Extraction Reagent that was used to resuspend the original cell paste. Pipet up and down and vortex to obtain an even suspension. Complete resuspension of the pellet will solubilize and remove contaminating proteins and is critical to obtaining a high purity preparation.
3. Add rLysozyme™ Solution to a final concentration of 1 KU/ml. Mix by gently vortexing and incubate at room temperature for 5 min.

Note: If using BugBuster Master Mix, additional rLysozyme is not required.

4. Add equal volume of 1:10 diluted BugBuster reagent (in deionized water) to the suspension and mix by vortexing for 1 min.
5. **Optional:** Add protease inhibitors. Protease inhibitors are compatible with all BugBuster formulations. Please see the *General considerations* section on p 4 for recommended protease inhibitors.
6. Centrifuge the suspension at 5,000 × g for 15 min at 4°C to collect the inclusion bodies. Remove the supernatant with a pipet.
7. Resuspend the inclusion bodies in 1:10 diluted BugBuster (10 ml per gram of original cell paste), mix by vortexing, and centrifuge as in step 6.
8. Repeat step 7 twice for a total of 3 washes with 1:10 diluted BugBuster.
9. Resuspend the pellet once more in 1:10 diluted BugBuster (10 ml per gram of original cell paste).
10. For SDS-PAGE analysis, remove a small sample of the suspension and combine with equal volume of 4X SDS Sample Buffer (Cat. No. 70607-3). Immediately heat for 3 min at 85°C to denature proteins and then store at –20°C until SDS-PAGE analysis.
11. Centrifuge the remaining IB suspension at 16,000 × g for 15 min at 4°C and remove the supernatant. Washed IB pellets may be stored at –80°C for several days.