

BugBuster™ Protein Extraction Reagent



Description

| | | |
|---|----------|---------|
| BugBuster Protein Extraction Reagent | 100 ml | 70584-3 |
| | 500 ml | 70584-4 |
| BugBuster 10X Protein Extraction Reagent | 10 ml | 70921-3 |
| | 50 ml | 70921-4 |
| BugBuster HT Protein Extraction Reagent | 100 ml | 70922-3 |
| | 500 ml | 70922-4 |
| BugBuster (primary amine-free) Protein Extraction Reagent | 100 ml | 70923-3 |
| | 500 ml | 70923-4 |
| BugBuster plus Benzonase® | 500 ml | 70750-3 |
| | 10,000 U | |

BugBuster Protein Extraction Reagent is formulated for the gentle disruption of the cell wall of *E. coli*, resulting in the liberation of soluble protein. It provides a simple, rapid, low cost alternative to mechanical methods such as French Press or sonication for releasing expressed target proteins in preparation for functional studies or purification. The proprietary formulation utilizes a mixture of non-ionic detergents that is capable of cell wall perforation without denaturing soluble protein.

BugBuster (primary amine-free) is intended for applications that are sensitive to the presence of primary amines, such as protein immobilization and crosslinking. BugBuster HT is a ready-to-use, premixed formulation of BugBuster and Benzonase® Nuclease ideally suited to high-throughput applications. BugBuster is also available as a 10X stock that can be diluted in the buffer system of your choice, subject to certain considerations (see below for details).

Benzonase is a genetically engineered endonuclease from *Serratia marcescens* (1, 2). 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 (3). The enzyme completely digests nucleic acids to 5'-monophosphate terminated oligonucleotides 2–5 bases in length (2, 4). Benzonase is ideal for a wide variety of applications where complete digestion of nucleic acids is desirable.

BugBuster Protein Extraction Reagent plus Benzonase Nuclease is an efficient combination for gently releasing target proteins and markedly reducing extract viscosity prior to downstream processing. Cells are harvested by centrifugation as usual, followed by suspension in BugBuster reagent at room temperature and Benzonase treatment. During a brief incubation, soluble proteins are released and nucleic acids digested. Insoluble proteins and cell debris are easily removed by centrifugation. The resulting low viscosity clarified extract contains soluble proteins ready for additional purification or analysis. The soluble fraction is directly compatible with most of the chromatography resins offered by Novagen. The insoluble fraction can be further processed to yield purified inclusion bodies.

Storage

Store BugBuster, BugBuster (primary amine-free), or BugBuster 10X Protein Extraction Reagent at room temperature.

Store BugBuster HT at 4°C.

Benzonase is supplied in 50% glycerol containing 50 mM Tris-HCl pH 8, 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 as freezing Benzonase results in loss of activity.

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Using BugBuster™ 10X

The following guidelines should be considered before diluting BugBuster 10X Protein Extraction Reagent for use.

- Certain components of BugBuster are unstable at acid 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. See note e) below for further details.
- BugBuster is compatible with phosphate, Tris and PIPPS buffer systems. Test a small aliquot of BugBuster 10X by dilution in other buffer systems to be sure that precipitation will not occur.

Using BugBuster HT

BugBuster HT is supplied as a ready-to-use reagent and can be used directly in the following protocols. Before use, check BugBuster HT for precipitation. If any cloudiness is present, brief warming of BugBuster HT will dissolve the precipitate. The presence of Benzonase® Nuclease in BugBuster HT should be considered if the desired protein must be nuclease-free; see note n) below for further details.

Using BugBuster (primary amine-free)

BugBuster (primary amine-free) is similar to the original formulation of BugBuster, and can be used directly in the following protocols, with or without addition of Benzonase® Nuclease.

Cell Extract Preparation

Soluble fraction

Note: This fraction will consist of soluble protein present in both the periplasm and cytoplasm. If a separate periplasmic fraction is desired, follow the osmotic shock procedure given in the pET System Manual (TB055) or other suitable method. The final pellet from the osmotic shock procedure can then be used in this protocol.

1. Harvest cells from liquid culture by centrifugation at $10,000 \times g$ for 10 min using a pre-weighed centrifuge tube. Decant and allow the pellet to drain, removing as much liquid as possible. Determine the wet weight of the pellet.
2. Completely resuspend the cell pellet in room temperature BugBuster reagent by pipetting or gentle vortexing, using 5 ml BugBuster per gram of wet cell paste. For small cell pellets (in 96 wells for example) use a volume of BugBuster equivalent to $1/20^{\text{th}}$ of the original bacterial culture volume pelleted. At this point protease inhibitors may be added if desired (see notes below).

Optional: Add $1 \mu\text{l}$ (25 units) of Benzonase per ml of BugBuster reagent used for resuspension.

3. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature. If Benzonase was added, the extract should not be viscous at the end of the incubation.
4. Remove insoluble cell debris by centrifugation at $16,000 \times g$ for 20 min at 4°C . If desired, save the pellet for inclusion body purification as described below.
5. Transfer the supernatant to a fresh tube. The soluble extract can be loaded directly onto most of Novagen's protein purification resins (and many other systems). Maintain clarified extracts on ice for short term storage (a few h) or freeze at -20°C until needed (see notes below).

Inclusion body purification

1. Process the induced culture according to steps 1–4 above for the soluble protein fraction.



2. Resuspend the pellet from step 4 above in the same volume of BugBuster reagent that was used to suspend the cell pellet. Pipet up and down and vortex to obtain an even suspension (see note *i* below).
3. Add lysozyme to a final concentration of 200 µg/ml (use 1/50 volume of a freshly prepared 10 mg/ml stock in water). Vortex to mix and incubate at room temperature for 5 min.
4. Add 6 volumes of 1:10 diluted BugBuster reagent (in deionized water) to the suspension and vortex for 1 min.
5. Centrifuge the suspension at 16,000 × g for 15 min at 4°C to collect the inclusion bodies. Remove the supernatant with a pipet.
6. Resuspend the inclusion bodies in ½ the original culture volume of 1:10 diluted BugBuster, mix by vortexing, and centrifuge as in step 5. Repeat this wash step 2 more times.
7. Resuspend the final pellet of purified inclusion bodies in your buffer of choice. Inclusion bodies prepared in this manner are compatible with resuspension in 1X IB Solubilization Buffer provided in Novagen's Protein Refolding Kit.

Notes

- a) The recommended amount of BugBuster to use (5 ml/g cells) corresponds to about 3 ml for a 50 ml culture, which usually produces about 0.5–0.7 g cell pellet under the conditions described here. 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, as required, to simplify handling.
- b) For small scale extractions (1.5 ml or less), centrifugation to remove cell debris and to collect inclusion bodies can be performed at top speed in a microcentrifuge (14,000–16,000 × g).
- c) Extraction and Benzonase digestion are most efficient when performed at room temperature. Protease inhibitors (e.g., Protease Inhibitor Cocktail Set III) can be added along with the BugBuster reagent if desired. It should also be noted that serine protease inhibitors should be used with caution if the target protein is to be treated with thrombin, Factor Xa or enterokinase, because any active inhibitor carried through the purification may affect cleavage reactions. Although it is likely that the inhibitors will be removed and/or inactivated during purification, we recommend including a dialysis or gel filtration step prior to proteolytic cleavage with rEK, Factor Xa or thrombin.
- d) BugBuster can be used for frozen or fresh cell pellets. There are instances where the processing of frozen cell pellets may be required, especially when monitoring expression levels over an extended time course or where the number of samples is large. BugBuster is fully compatible with frozen cell pellets; in fact, more protein may be released if cells are frozen prior to processing. When performing comparisons of target protein from multiple samples, all samples should be processed in a similar manner (i.e. all fresh or all frozen) to prevent erroneous results. Superior extraction efficiencies may be obtained when pelleted cells are frozen prior to resuspension in BugBuster and processing. Extraction efficiency is somewhat strain-dependent, and appears to be especially efficient with BL21 and derivative strains (e.g., Tuner™ strains).
- e) BugBuster has known compatibility with Tris, PIPPS and phosphate-based buffer systems in the near-neutral pH range. The detergents in BugBuster will precipitate at or above 1 M NaCl. When using high salt buffer compositions or acid or alkaline pH ranges for chromatography, BugBuster should be evaluated on a small-scale prior to scale-up of purification procedures. Attention should be paid to any observed precipitation of the BugBuster reagent or target protein, and compatibility of the BugBuster reagent with buffers used for equilibration of chromatography columns.
- f) BugBuster reagent is compatible with reducing agents such as 2-mercaptoethanol and DTT, as well as with EDTA and many other compounds. Note, however, that reducing agents may activate certain proteases, and reducing agents and EDTA will interfere with protein binding to His•Bind Resin. Up to 20 mM 2-mercaptoethanol can be used with Ni-NTA His•Bind Resin. Benzonase is minimally inhibited by EDTA concentrations < 1 mM. However, an EDTA concentration of 5 mM results in a 90% loss of activity due to chelation of essential Mg²⁺ ions.



BugBuster™ Protein Extraction Reagent

- g) Extracts should be stored at a temperature that is compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.
- h) Storage at temperatures below 4°C may cause precipitation of the detergents in BugBuster reagent. Incubate in a room temperature water bath with gentle swirling to redissolve.
- i) Thorough resuspension of pellets is critical to obtaining a high purity inclusion body preparation. The inclusion body pellet must be completely dispersed during BugBuster wash steps in order to solubilize and remove contaminating proteins from the pellet.
- j) For SDS-PAGE and Western blot analysis, a load volume of approximately 2.4 µl would give a normalized amount of protein, given a concentration factor of 25 and an OD₆₀₀ of 3 at harvest, using a 15 well mini gel. When 1/5 culture volume of BugBuster is used, this would correspond to a load volume of approximately 12 µl (since the concentration factor is 5). Because the optimal amount of material to load will vary with the expression level of the target protein, the efficiency of the extraction, and detection sensitivity of the Western blot method, these amounts should be used as guidelines only.
- k) Benzonase can be diluted for ease of handling small quantities with 50 mM Tris-HCl pH 8, 20 mM NaCl, and 2 mM MgCl₂. Diluted samples can be stored at 4°C for several days without loss of activity.
- l) Although Benzonase requires Mg²⁺ for activation, it does not appear to require additional Mg²⁺ under the conditions described here. The activity is sufficient for effective viscosity reduction and nucleic acid digestion using just the BugBuster reagent for cell resuspension.
- m) 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.
- n) 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 by anion exchange chromatography. Residual nuclease activity can be checked by incubation of the purified protein with RNA or DNA markers followed by gel analysis.
- o) Benzonase appears to have no effect on the FRETWorks™ S•Tag™ Assay.

Related Products

| Product | Size | Cat. No. |
|--|------------------|--------------------|
| Protease Inhibitor Cocktail Set II (with EDTA) | | 539132 |
| Protease Inhibitor Cocktail Set III (without EDTA) | | 539134 |
| Protein Refolding Kit | | 70123-3 |
| Benzonase Nuclease, Purity > 99% | 10,000 U | 70664-3 |
| Benzonase Nuclease, Purity > 90% | 10,000 U | 70746-3 |
| BugBuster Protein Extraction Reagent | 100 ml 500 ml | 70584-3 70584-4 |

References

1. Eaves, G. N. and Jeffries, C. D. (1963) *J. Bact.* **85**, 273–278.
2. Nestle, M. and Roberts, W. K. (1969) *J. Biol. Chem.* **244**, 5219–5225.
3. (1999) Benzonase® Brochure, Code No. W 220911, Merck KGaA, Darmstadt, Germany.
4. Janning, P., Schrader, W. and Linscheid, M. (1994) *Rapid Commun. Mass Spectrom.* **8**, 1035–1040.