Lysonase™ Bioprocessing Reagent

Description

Lysonase Bioprocessing Reagent	0.2 ml	71230-3
	1 ml	71230-4
	5 × 1 ml	71230-5

Description

Lysonase Bioprocessing Reagent is an optimized, ready-to-use blend of rLysozyme™ Solution and Benzonase® Nuclease. rLysozyme Solution (see Technical Bulletin 334) is a highly purified and stabilized recombinant lysozyme with a specific activity 250 times greater than that of chicken egg white lysozyme (1). Benzonase Nuclease (see Technical Bulletin 261) is a genetically engineered non-specific endonuclease from *Serratia marcescens* (2, 3) that degrades all forms of DNA and RNA (single stranded, double stranded, circular, linear), reducing protein extract viscosity, and increasing protein yield (4). The combined activities of rLysozyme and Benzonase Nuclease significantly increase protein extraction efficiency and overall protein yield. Lysonase facilitates downstream processing of protein extracts in procedures such as chromatography, ultrafiltration, robotic liquid handling, and electrophoresis.

This protocol describes *E. coli* lysis with Lysonase Bioprocessing Reagent treatment combined with a freeze/thaw of the cell pellet. Protocols for *E. coli* lysis using Lysonase with mechanical lysis, BugBuster®, and PopCulture™ Protein Extraction Reagents are described in Techncial Bulletins 054 (His•Bind® Kits), 245 (BugBuster Protein Extraction Reagent) and 323 (PopCulture Reagent). PopCulture extraction using Lysonase and purification of His•Tag® of GST•Tag™ fusion proteins in a 96-well format can be achieved with RoboPop™ Purification Kits (see Technical Bulletins 327 and 346). PopCulture extraction using Lysonase is also utilized in the RoboPop Solubility Screening Kit for high throughput (HT) screening of solubility optimization experiments (see Techncial Bulletin 362).

Components

0.2 <u>or</u> 1.0 <u>or</u> 5 × 1ml	Lysonase Bioprocessing Reagent (in 50 mM Tris-HCl, 20 mM NaCl, 2 mM $MgCl_2$, 50% glycerol, pH 8.0)

Storage

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

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E. Coli Lysis using Freeze/Thaw

This protocol isolates the soluble protein from the periplasm and cytoplasm using Lysonase Bioprocessing Reagent and a freeze/thaw of the cell pellet. Note that if a periplasmic fraction is desired, the osmotic shock procedure provided in the pET System Manual (Technical Bulletin 055) should be followed. 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 × 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. Freeze the pellet completely at –20°C or –70°C.
3. Completely thaw and resuspend the frozen cell pellet by pipetting up and down or gentle vortexing in room temperature lysis buffer (50 mM Tris-HCl or NaH_2PO_4, pH 7–8, 5% glycerol, 50 mM NaCl) using 7 ml lysis buffer per gram of wet cell paste. Although protease inhibitors are often unnecessary, they can be added to protect against degradative enzymes. 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 (rEK) (Cat. No. 69066-3) because active inhibitor carried through the purification may affect cleavage reactions. Include dialysis or gel filtration prior to proteolytic cleavage with rEK, Factor Xa, or thrombin if serine protease inhibitors are used. If proteolytic cleavage is a problem, try adding AEBSF (10–100 µM: Calbiochem Cat. No. 101500); Pepstatin A (1 µM; Calbiochem Cat. No. 516482); Leupeptin (10–100 µM; Calbiochem Cat. No. 108975); Aprotinin (2 µg/ml; Calbiochem Cat. No. 616398); E-64 (15 µg/ml; Calbiochem Cat. No. 324890); or Protease Inhibitor Cocktail Set II (with EDTA; Calbiochem Cat. No. 539132) or III (without EDTA, Calbiochem Cat. No. 539134). Note that Protease Inhibitors containing EDTA are not compatible with His•Bind® Resin.

Note: *DO NOT add Lysonase Bioprocessing Reagent until a uniform cell suspension has been obtained. The freeze/thaw step ruptures the cell membrane allowing rLysozyme to access the cell wall. If Lysonase is added prematurely, the immediate viscosity increase will make complete cell resuspension difficult and incomplete lysis may result.*

4. Add approximately 3 µl Lysonase per 1 ml lysis buffer (20 µl/gram cell paste).
5. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature. The extract should not be viscous at the end of the incubation. Longer incubation time may be required if lysis is performed at 4°C. Determine empirically.
6. Remove insoluble cell debris by centrifugation at 16,000 × g for 20 min at 4°C.
7. Transfer the supernatant to a fresh tube for analysis and/or purification. The extract can be loaded directly onto any Novagen protein purification resin (and numerous other systems). Maintain clarified extracts on ice for short-term storage (a few hours) or freeze at –20°C until needed.
8. If desired, save the pellet for inclusion body purification. See Technical Bulletin 055 for relevant protocols. Target proteins found in inclusion bodies that contain a His•Tag® sequence may be purified under denaturing conditions as described in Techncial Bulletin 054. Inclusion bodies can be solubilized and refolded prior to purification as described in Technical Bulletin 234.

United States & Canada 800-207-0144
Germany 0800 6931 000
United Kingdom 0800 622935
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www.novagen.com

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