

Sonicase™ Non-detergent Protein Extraction Reagent



Cat. Nos. R1013E, R1014E, & R1014S

DESCRIPTION

Sonicase Non-detergent Protein Extraction Reagent is an optimized mixture of Lyse-Aid™ Recombinant Lysozyme and Benzonase® Nuclease designed for convenient extraction of proteins from *E. coli*. The combined activities of Lyse-Aid and Benzonase simultaneously lyse the cells and degrade nucleic acids, resulting in a non-viscous lysate containing intact proteins.

When added to a suspension of frozen and thawed *E. coli* cells, Sonicase alone will extract soluble proteins in the absence of detergent, sonication, or other mechanical disruption method.

Sonicase may also be combined with the non-ionic detergent-based SembaSonic™ Protein Extraction Reagent for extremely efficient preparation of cell lysates without the freeze-thaw step.

In either application Sonicase enables gentle and efficient extraction of proteins from milligrams to grams of cells without mechanically generated heat, shearing, or oxidative denaturation.

PROTEIN EXTRACTION USING SONICASE WITH FROZEN AND THAWED CELLS

The freeze-thaw step ruptures the cell membrane allowing the Lyse-Aid enzyme present in Sonicase access to the cell wall. In the recommended protocol below, pelleted cells are first resuspended in buffer before freezing. If cell pellets are frozen first and then resuspended in buffer plus Sonicase, extraction efficiency will be significantly lower.

1. Collect cells by centrifugation at 9,000 x g for 15 min in a tared tube or bottle. Remove as much supernatant as possible and determine the weight (mass) of the pellet.
2. Thoroughly resuspend the cell pellet in lysis buffer (50 mM Tris-Cl, 50 mM NaCl, pH 7.5) using 7 ml per gram of cell paste and mix by pipetting up and down. If required, EDTA or other protease inhibitors can be added to the buffer. Generally a ratio of 100 µl lysis buffer/ml of the original culture volume will give good results.
3. Freeze the resuspended cells completely at -20°C or lower temperature. Rapid freezing can be accomplished using a dry ice-ethanol bath.
4. Completely thaw the frozen cell suspension at room temperature, mixing to ensure that the solution is homogeneous.
5. Add 3 µl Sonicase per ml cell suspension (20 µl per gram cell paste). **IMPORTANT:** Do not add Sonicase until a uniform cell suspension has been obtained. If Sonicase is added prematurely, partial lysis may result.

6. Incubate the mixture on a shaking platform or rotating mixer at a slow setting for 20 min at room temperature. The lysate should appear non-viscous.
7. Centrifuge at 16,000 x g for 20 min at 4°C. Shorter centrifugation times and lower g forces may be used as long as lysate clarification is adequate.
8. Transfer the supernatant, which contains the extracted soluble proteins, to a fresh tube. The pellet can be further processed to purify inclusion bodies.

NOTE: Soluble protein recoveries using a single freeze-thaw-Sonicase treatment are typically 40-50% of those obtained using Sonicase plus SembaSonic Protein Extraction Reagent or SembaSonic Master Mix (next section). Total protein extraction efficiency may be improved by performing additional freeze-thaw cycles prior to the addition of Sonicase.

PROTEIN EXTRACTION USING SONICASE WITH SEMBASONIC™ PROTEIN EXTRACTION REAGENT

Sonicase can be used in combination with SembaSonic Protein Extraction Reagent (Cat. Nos. R1011E, R1012E) for convenient preparation of *E. coli* protein extracts without the need for freeze-thaw or mechanical disruption. SembaSonic contains a non-ionic detergent that normally does not interfere with protein activity. For each gram of cell paste prepared as in Step 1, resuspend in 5 ml 1X SembaSonic reagent containing 10 µl Sonicase, and incubate 10-20 minutes at room temperature. Another option for convenience is to use SembaSonic Master Mix (Cat. Nos. R1016E, R1017E), which is a pre-mixed combination of all three of the reagents: Benzonase, Lyse-Aid, and SembaSonic Protein Extraction Reagent, in 25 mM Tris-Cl, pH 8.0.

STORAGE

Store at -20°C. Sonicase is supplied in 50% glycerol, 50 mM Tris-Cl, 20 mM NaCl and 2 mM MgCl₂, pH 8.0. The enzyme preparation is stable for 2 years when stored at -20°C.

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