

SembaSonic™ 10X Protein Extraction Reagent



Cat. Nos. R1011E & R1012E

DESCRIPTION

SembaSonic 10X Protein Extraction Reagent is a proprietary concentrate containing a non-ionic detergent designed for preparation of cell lysates from *E. coli*. When used at a 1X final concentration, soluble proteins are gently released without denaturation or interference with activity. When combined with a buffer such as Tris or phosphate, Lyse-Aid™ Recombinant Lysozyme, and Benzonase® Nuclease, bacterial cell walls are effectively disrupted and nucleic acids are degraded to produce a non-viscous protein extract suitable for assays or chromatography. SembaSonic provides an easy, fast and economical substitute for physical disruption such as sonication or French Press homogenization. SembaSonic is ideal for rapid preparation of bacterial cell lysates for purification of recombinant proteins with Semba's Octave™ Chromatography System.

CELL LYSIS WITH SEMBASONIC PROTEIN EXTRACTION REAGENT

The following guidelines should be considered before dilution of SembaSonic 10X reagent:

- SembaSonic 1X reagent is generally used with Tris- and phosphate-based buffer systems in the neutral pH range of 7.0 to 8.0. However it can be used with buffer systems from pH 5.0 to 10.0.
- The salt concentration should not exceed 1.0 M. In addition, note that monovalent cation concentrations above 150 mM inhibit Benzonase® Nuclease activity.
- Protease inhibitors and EDTA are compatible with SembaSonic reagent. Concentrations of EDTA higher than 1 mM can interfere with Benzonase treatment and IMAC purification.
- SembaSonic reagent can be used with reducing reagents such as 2-mercaptoethanol and dithiothreitol. Note that these reducing agents may interfere with IDA-based metal affinity chromatography.

An abbreviated protocol for the use of SembaSonic reagent in preparation of protein samples from *E. coli* extracts is given here.

1. Collect cells by centrifugation at 9000 x g for 10 min in a tared tube or bottle. Remove as much supernatant as possible and determine the weight (mass) of the pellet. The cells can be prepared fresh or previously frozen.
2. For each gram of cell paste, prepare 5 ml 1X SembaSonic in a desired buffer such as 25 mM Tris-Cl, 50 mM NaCl, 5% glycerol, pH 8.0. This solution should be prepared at room temperature.
3. Resuspend the pellet using 5 ml 1X SembaSonic per gram of cells. Small volumes can be mixed by vortexing; for larger volumes swirl the solution and use a

pipet to stir and pipet the cells up and down until the solution is homogeneous. Perform this step at room temperature.

4. Incubate the mixture on a shaking platform or rotating mixer at a slow setting for 10-20 min at room temperature.
5. Centrifuge at 16,000 x g for 20 min at 4°C.
6. Transfer the supernatant, which contains the extracted soluble proteins, to a fresh tube. The pellet can be further processed to purify inclusion bodies.

ADDITIVES THAT IMPROVE HANDLING AND EFFICIENCY OF EXTRACTION

Addition of Lyse-Aid™ Recombinant Lysozyme (Cat. Nos. R1003E & R1004E) improves the efficiency of extraction with SembaSonic reagent. Add 5 KU Lyse-Aid per 5 ml SembaSonic 1X reagent prepared in Step 2.

To reduce viscosity of the cell extract by degradation of nucleic acids, add Benzonase® Nuclease (Cat Nos. R1005E, R1006E, R1007E, R1008E, R1009E & R1010E). Add 125 units of Benzonase per 5 ml 1X SembaSonic reagent prepared in step 2. (Note that although Benzonase requires Mg²⁺ for activation, it does not require additional Mg²⁺ for the conditions described in Steps 2-6.)

Another alternative is to add Sonicase™ Non-Detergent Protein Extraction Reagent (Cat Nos. R1013E, R1014E & R1014S). Sonicase is an optimized mixture of Benzonase and Lyse-Aid enzymes. Add 10 µl Sonicase per 5 ml SembaSonic 1X reagent prepared in Step 2.

STORAGE

Store SembaSonic 10X Protein Extraction Reagent at room temperature.

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