

SembaSonic™ Master Mix



Cat. Nos. R1016E & R1017E

DESCRIPTION

SembaSonic™ Master Mix is designed for convenient, efficient extraction of proteins from *E. coli*. This all-in-one reagent contains an optimized mixture of SembaSonic Protein Extraction Reagent, Lyse-Aid™ Recombinant Lysozyme, and Benzonase® Nuclease. Virtually complete cell lysis is accomplished by the combination of gentle non-ionic SembaSonic detergent and Lyse-Aid enzyme, with simultaneous degradation of nucleic acids by Benzonase to remove interference and reduce viscosity. Because there is no need for dilution or separate addition steps, SembaSonic Master Mix is well-suited for processing multiple samples in parallel and provides absolute consistency from experiment to experiment.

Protein extracts prepared with SembaSonic Master Mix are compatible with the BCA protein assay, many reporter assays, and chromatography matrices (e.g. affinity, size exclusion, ion exchange). The formulation contains 25 mM Tris-Cl, pH 8.0.

The two available package sizes provide sufficient reagent to process 20 g and 100 g cell paste.

CELL LYSIS WITH SEMBASONIC MASTER MIX

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. Resuspend the cell pellet using 5 ml room temperature SembaSonic Master Mix per gram of cells. This volume typically corresponds to a ratio of about 2.5 ml Master Mix per 50-ml culture. For small cultures use up to 1/5 culture volume for resuspension (e.g., use 300 µl SembaSonic Master Mix for 1.5-ml cultures). There are no adverse effects of using higher volume ratios.

Small volumes can be mixed by gentle vortexing. For larger volumes use a pipet to stir and pipet the cells up and down and swirl until the solution is homogeneous.

3. Incubate the mixture on a shaking platform or rotating mixer at a slow setting for 10-20 min at room temperature. The extract should not be viscous after incubation.
4. Centrifuge at 16,000 x g for 20 min at 4°C.
5. Transfer the supernatant containing the extracted proteins, to a fresh tube. The pellet can be further processed to purify inclusion bodies.

The soluble extract can be loaded directly on most chromatography resins, stored for a few hours on ice, or frozen at -20°C or below for longer periods. Note that some target proteins may be inactivated by freeze-thaw cycles.

NOTES

- Protease inhibitors may be added if desired; however, certain inhibitors such as EDTA or serine protease inhibitors may affect downstream applications such as metal affinity chromatography or site-specific protease cleavage of fusion proteins.
- Protein precipitation of detergent-containing extracts using ammonium sulfate is altered by detergent effects on protein hydrophobicity.
- SembaSonic Master Mix contains Benzonase® Nuclease and may not be suitable for preparation of proteins expected to be nuclease-free.

STORAGE

Store SembaSonic Master Mix at 4°C. Do not freeze. If precipitation is observed, redissolve the precipitate by incubation at room temperature with gentle swirling prior to use.

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