

# Benzonase<sup>®</sup> Nuclease



Cat. Nos. R1005E, R1006E, R1007E, R1008E, R1009E, R1010E

## DESCRIPTION

Benzonase is ideal for a variety of applications such as purification of recombinant proteins from cell extracts where complete digestion of nucleic acids is desirable. It is the only known endonuclease which degrades all types of nucleic acids and therefore will give maximum viscosity reduction in cell extracts. This promiscuous endonuclease, which consists of two subunits of 30 kDa each, attacks and degrades all forms of DNA and RNA (single stranded, double stranded, linear and circular) and is effective over a wide range of conditions. Upon complete digestion, all free nucleic acids present in solution are reduced to 5'-monophosphate terminated oligonucleotides 2-5 bases in length. This enzyme is a genetically engineered endonuclease from *Serratia marcescens* produced and purified from an *E. coli* strain. Benzonase is available in two grades, purity > 99% and purity > 90%. Both preparations possess exceptionally high specific activities and are supplied free from measurable protease activities. Both grades are available at 25 U/μl or at high concentration of 250 U/μl.

## USING BENZONASE TO REDUCE VISCOSITY

Benzonase is effective over a wide range of conditions. For example, it is functional between pH 6 and 10 (optimal pH 8-9), from 0-42°C (optimal 37°C) and 1-10 mM Mg<sup>2+</sup> (optimal 1-2 mM) for activation. The enzyme is also active in the presence of ionic and nonionic detergents, PMSF (1mM), EDTA (1 mM), and urea (relative activity depends on specific conditions). Activity is inhibited by >150 mM monovalent cations (Na<sup>+</sup>, K<sup>+</sup>, etc.), > 100 mM phosphate, > 100 mM ammonium sulfate, or > 100 mM guanidine HCl.

Benzonase may be used with all methods of cell lysis, including detergent based methods, lysozyme treatment, freeze-thawing procedures and high-pressure homogenization. Viscosity reduction will occur significantly faster when Benzonase is added before lysis rather than post lysis. Typically 200-500 units of Benzonase are used per gram of cell paste. The efficiency of viscosity reduction may vary with buffers, cell types and cell lysis methods used. An abbreviated protocol for viscosity reduction in *E. coli* extracts is given here.

1. Collect cells by centrifugation at 5000 x g for 5 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, suspend in 5 ml 50 mM Tris-HCl pH 8.0, 50 mM NaCl, 5% glycerol, 0.5 mM dithiothreitol. If these buffer conditions are incompatible for downstream manipulations, add more Benzonase to compensate for sub-optimal activity. (Note that although Benzonase requires Mg<sup>2+</sup> for activation, it does not require additional Mg<sup>2+</sup> under many conditions.)

3. For each gram of cell paste, add 250 units Benzonase.
4. Lyse the cells by method of choice. This can be done most easily with the addition of SembaSonic™ Protein Extraction Reagent and Lyse-Aid™ Lysozyme (see below). For each gram of cell paste, add 0.5 ml 10X SembaSonic reagent and 5 KU Lyse-Aid.
5. Incubate the mixture on a shaking platform or rotating mixer at a slow setting for 10-20 min at room temperature. After this incubation, the cell extract should not be viscous.
6. Centrifuge at 16,000 x g for 20 min at 4°C.
7. Transfer the supernatant, which contains the extracted soluble proteins, to a fresh tube. The pellet can be further processed to purify inclusion bodies.

## USE WITH SEMBASONIC™ PROTEIN EXTRACTION REAGENT AND LYSE-AID™ RECOMBINANT LYSOZYME

Benzonase is ideal for use in combination with SembaSonic Protein Extraction Reagent (Cat. Nos. R1011E, R1012E) and Lyse-Aid (Cat. Nos. R1003E, R1004E) for convenient preparation of *E. coli* protein extracts without the need for mechanical disruption. For each gram of cell paste prepared as in Step 1, resuspend in 5 ml 1X SembaSonic reagent containing 25 mM Tris-HCl, pH 8.0, 125 U Benzonase, and 5 KU Lyse-Aid, 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 buffer, pH 8.0.

## STORAGE

Benzonase Nuclease is supplied in 50% glycerol containing 50 mM Tris-HCl pH 8.0, 20 mM NaCl and 2 mM MgCl<sub>2</sub>. The enzyme preparation is stable for 2 years when stored at -20°C. Benzonase can be diluted with 50 mM Tris-HCl, 20 mM NaCl, 2 mM MgCl<sub>2</sub>, pH 8.0. Diluted samples can be stored at 4°C for several days without loss of activity.

## UNIT DEFINITION

One unit of Benzonase Nuclease is defined as the amount of enzyme that causes a ΔA<sub>260</sub> of 1.0 in 30 min, which corresponds to complete digestion of 37 μg DNA.

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