TECHNICAL ARTICLE
January 2016

Bacterial Cell Lysis Buffer

At NZYTech we intend to develop, manufacture and market solutions that simplify, accelerate and improve life sciences research. Thus, conscious of the pressures that befall on structural genomics and proteomics as a result of the dramatic increasing number of genome sequences available nowadays, we have recently developed a high-throughput (HTP) platform to efficiently clone and express a large amount of recombinant proteins that can be rapidly used in subsequent protocols for function and structure determination. When implementing a HTP platform for production of hundreds of proteins at the same time it is, obviously, necessary to modify the *modus operandi* of the cloning, expression and production protocols. So, the conventional cell lysis using mechanical procedures such as French Press or sonication, even adapted for HTP approaches, is not practical and economical and the solution is to use ready-to-use chemicals for simultaneous bacterial cell walls disruption. Here, we would like to introduce an innovative reagent for the gentle disruption of *Escherichia coli* cell wall, generating a homogeneous cell-free extract. Even for laboratories studying a single protein target, such as designed proteins, the usage of this product is beneficial as the yield of extracted protein will be significantly improved besides it is less time-consuming than the mechanical lysis.

NZY Bacterial Cell Lysis Buffer (Cat. No. MB1780) is a Tris-buffered formulation (pH 7.5) with lysozyme and DNase I provided separately for more convenient use. The addition of these enzymes allows a most efficient extraction, however for some over-expressed proteins and particular *E. coli* strains the addition of lysozyme may not be required. Furthermore, the presence of lysozyme and DNase I might interfere with some downstream applications, so in these situations the addition of these enzymes should be omitted. Additional components, such as protease inhibitors, salts, reducing agents and chelating agents (not provided), may be added to the lysate obtained using the NZY Bacterial Cell Lysis Buffer, depending on the particular application.

The efficiency of the chemical lysis provided by NZY Bacterial Cell Lysis Buffer was compared to a mechanical procedure (sonication) in a test with 22 cultures containing recombinant *Escherichia coli* BL21(DE3) expressing different proteins from the anaerobic ruminal bacterium *Ruminococcus flavefaciens* (P1-P22). All *E. coli* cultures containing recombinant plasmids were cultured, in duplicate, in 5 mL of NZY Auto-Induction LB medium (NZYTech, Cat. No. MB179) supplemented with appropriate antibiotic. After growth and induction at 30 °C during 16h, cells were harvest at 5,000 ×g. Each cell-pelleted culture was lysed, in parallel, by sonication and chemical lysis provided by NZY Bacterial Cell Lysis Buffer. Recombinant proteins extracted using these two strategies were purified through Immobilized Metal Affinity

Chromatography (IMAC) and separated by SDS-PAGE. The levels of protein obtained were evaluated (Figure 1). In general, the data revealed that NZY Bacterial Cell Lysis Buffer extracts shows higher or similar levels of target protein when compared with sonication. A detailed analysis of data collected reveals that for only four proteins in test, sonication performed slightly better than the NZYTech's protein extraction reagent. Besides the high levels of recombinant proteins obtained after cell lysis with the NZY Bacterial Cell Lysis Buffer, this procedure offers a rapid and less labor-intensive strategy than sonication, when purifying multiple proteins in parallel.

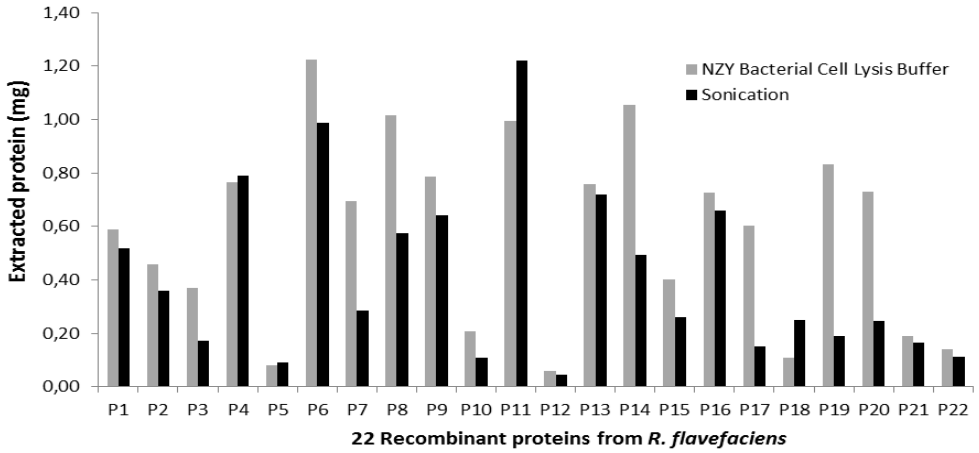
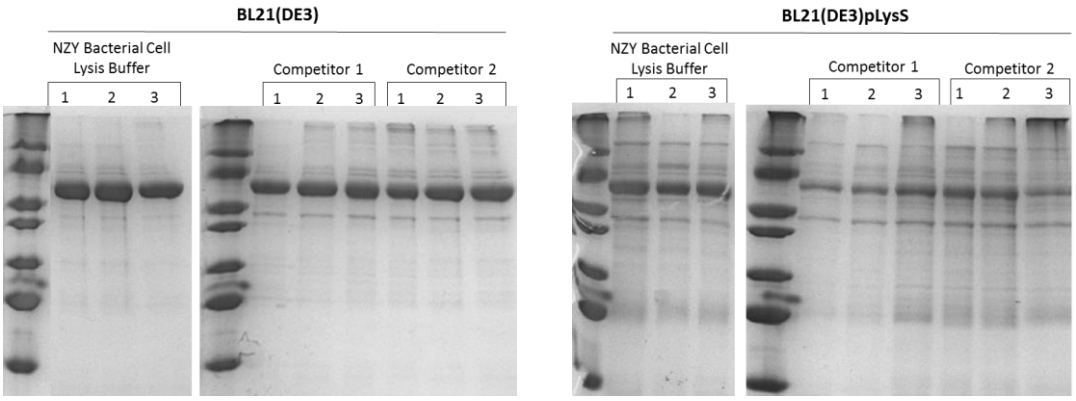


Figure 1. Levels of extracted protein obtained from 22 different recombinant *E. coli* BL21(DE3) cultures using NZY Bacterial Cell Lysis Buffer or a mechanical procedure (sonication). The 22 recombinant *Ruminococcus flavefaciens* proteins were purified through IMAC and levels of protein obtained evaluated.

The NZY Bacterial Cell Lysis Buffer can be used to disrupt the most common bacterial host strains and it is especially efficient with BL21 strain and its derivatives. The use of pLysS or pLysE hosts can enhance the extraction procedure since lysozyme is endogenously produced. We tested NZY Bacterial Cell Lysis Buffer for the extraction of a prokaryotic recombinant protein in two expression *E. coli* strains: BL21(DE3) and BL21(DE3)pLysS. For comparison, two competitor's bacterial cell lysis reagents (competitors 1 and 2) were used following the manufacturer's recommendations. Recombinant proteins were purified through IMAC and separated by SDS-PAGE (see Figure 2). The data revealed that yields of extracted protein using NZY Bacterial Cell Lysis Buffer is higher than those obtained using the competing products when expressing proteins in *E. coli* BL21(DE3)pLysS. When using *E. coli* BL21(DE3) as host, yields of extracted protein are slightly higher when compared with the competitor's product. Complete cell lysis by NZY Bacterial Cell Lysis Buffer occurred around 10 minutes after incubation at room temperature with shaking in an orbital shaker incubator. The time for complete resuspension of cell pelleted extract was similar using the three reagents in study (data not show). Functional activity of the protein in test was evaluated showing not be affected by the chemical lysis procedures (data not show).

A)



B)

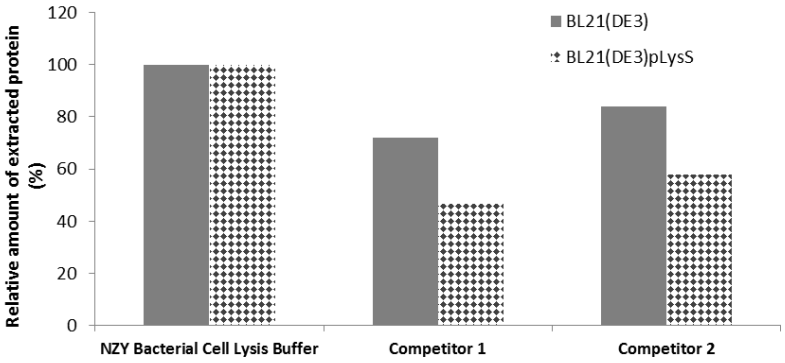


Figure 2. Comparing the efficiency of cell lysis using three different protein extraction reagents. *E. coli* cells harvested from 5 mL of cultured media were lysed (in triplicates) using three different protein extraction products: NZY Bacterial Cell Lysis Buffer, Competitor 1, and Competitor 2. **A)** The recombinant protein was purified through IMAC and separated through SDS-PAGE. **B)** Levels of extracted protein obtained were evaluated.