Auto-Induction Systems

At NZYTech we are constantly developing innovative products and services in our main areas of expertise, aiming to achieve the highest standards for laboratory research and industrial analysis. We intend to serve diligently the scientific community and to provide satisfying solutions to customers worldwide. With this in mind we have recently developed a high-throughput (HTP) platform to efficiently generate hundred to thousands of expressing clones, thus leading to the rapid characterization and screening of recombinant proteins that can be applied in subsequent protocols from structure determination to industrial procedures. To achieve this, we have optimized, in a high-throughput away, most of the classical and standard methods used in molecular biology. Here we would like to introduce two innovative *Escherichia coli* auto-induction methods to grow cells to high densities and to achieve high levels of recombinant protein expression with IPTG-inducible bacterial expression systems. The use of high-throughput methods to assign genomic sequence data that is continuously growing requires rapid, simple, efficient and accurate techniques allowing the manipulation of multiple clones, cultures, genes and proteins at the same time. So, the development of auto-induction media for *E. coli* will allow the production of a large number of recombinant strains in parallel and in a highly effective way. In addition, even when producing a limited number of recombinant proteins in *E. coli* the usage of these media is beneficial as the levels of protein yield will be significantly improved due to the high cell densities afforded by the media.

NZY Auto-Induction LB medium (powder) (Cat. No. MB179) is an innovative culture medium that contains all components required to support high cell densities and controllable gene induction. The method is based on the presence of different carbon sources that are metabolized differentially to promote culture growth to high cell densities and subsequently induce protein expression from lac-based promoters. This offers great convenience allowing high cell densities and spontaneous gene induction without monitoring cell grow, saving you more time to perform other tasks. In contrast, NZY Auto-Induction Kit (Cat. No. MB180) is an innovative product to supplement your specific *E. coli* culture medium. The kit contains all required supplements to promote bacterial grow to high cell densities while supporting auto-induction of gene expression. NZY Auto-Induction Kit contains two concentrated sterile solutions and was designed for IPTG-inducible bacterial expression systems. Addition of these solutions to traditional complex media, such as Luria-Bertani (LB) broth or Terrific Broth (TB), results in high-level protein production. The kit simplifies induction of expression constructs by eliminating the need to take repeated OD readings and IPTG addiction. Like the NZY Auto-Induction LB medium (powder), this method is also based on medium components that are metabolized differentially to promote culture growth to high cell densities and subsequent
induction of protein expression from lac-based promoters. Both systems are ideal for high-throughput methods, when you have to grow multiple cultures expressing various proteins simultaneously.

NZY Auto-Induction LB medium was tested for growing two different recombinant E. coli BL21(DE3) strains expressing different proteins (Protein A and B) and compared with a competitor product. The data, presented in Figure 1, reveals that induction starts at OD600nm of ≈ 8 (after around 6h of incubation in 50 mL cultured flasks at 37 °C) and maximal levels of recombinant proteins were obtained at OD600nm values of 13-14. Levels of protein expression obtained using NZYTech medium are equivalent or slightly higher when compared with a competitor auto-induction product.

![Figure 1. The levels of expression of two recombinant proteins (A and B) were tested when E. coli BL21(DE3) strains were grown in NZY Auto-Induction LB medium (powder) or a Competitor Auto-Induction medium. Samples were taken at different time points (1-7) during growth to construct growth curves (I) and the corresponding cell extracts were separated through SDS-PAGE (II). M: Low Molecular Weight (LMW) Protein Marker (MB082).](image)

We tested NZY Auto-Induction LB medium for the production in parallel of 24 recombinant proteins from the anaerobic ruminal bacterium Ruminococcus flavefaciens in two expression E. coli strains: BL21(DE3) and BL21(DE3)pLysS. For comparison, a competitor auto-induction medium was used following the manufacturer recommendations. Recombinant proteins were purified through Immobilized Metal Affinity Chromatography (IMAC) and separated by SDS-PAGE (see Figures 2 and 3). In general, the data revealed that NZYTech medium shows similar or higher levels of pure protein when compared with the competitor product. A detailed analysis of data collected shows that for 13 proteins in test, NZYTech auto-induction medium performed better than the competitor. Only one protein (protein number 20) was shown to be produced with higher levels using the competitor auto-induction medium. For the remaining 10 proteins, the differences observed between media were not significant.
Figure 2. Levels of purified protein obtained from 24 different recombinant *E. coli* BL21(DE3) strains grown in NZY Auto-Induction LB medium (powder) or in a Competitor Auto-Induction medium. The 24 recombinant *Ruminococcus flavefaciens* proteins were purified through IMAC and levels of protein obtained evaluated (A) while the degree of purification was confirmed through SDS-PAGE (B). M: Protein Marker.

Figure 3. Levels of purified protein obtained from 24 different recombinant *E. coli* BL21(DE3)pLysS strains grown in NZY Auto-Induction LB medium (powder) or in a Competitor Auto-Induction medium. The 24 recombinant *Ruminococcus flavefaciens* proteins were purified through IMAC and levels of protein obtained evaluated (A) while the degree of purification was confirmed through SDS-PAGE (B). M: Protein Marker.
Finally, a range of experiments were performed to compare the efficiency of NZY Auto-Induction LB medium (powder) and NZY Auto-Induction Kit. The data, presented in Figures 4 and 5, reveal that both media performed at very high levels both in terms of cell density and recombinant protein expression.

Figure 4. Levels of protein purified from 24 different recombinant *E. coli* BL21(DE3) strains grown using the NZY Auto-Induction Kit as supplement of LB media and the NZY Auto-Induction LB medium (powder; MB179). The 24 recombinant *Ruminococcus flavefaciens* proteins were purified through IMAC and levels of protein obtained evaluated (A) while the degree of purification was confirmed through SDS-PAGE (B). M: Low Molecular Weight (LMW) Protein Marker (MB082).

Figure 5. Levels of protein purified from 24 different recombinant *E. coli* BL21(DE3)pLysS strains grown using the NZY Auto-Induction Kit as supplement of LB media and the NZY Auto-Induction LB medium (powder; MB179). The 24 recombinant *Ruminococcus flavefaciens* proteins were purified through IMAC and levels of protein obtained evaluated (A) while the degree of purification was confirmed through SDS-PAGE (B). M: Low Molecular Weight (LMW) Protein Marker (MB082).