

## How can we help you?

# Answers to questions about the Overnight Express™ Autoinduction Systems



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### What is autoinduction?

Autoinduction refers to bacterial cultures grown in media containing specific components that, after an initial period of tightly-regulated, uninduced growth, automatically induce target protein expression, without IPTG. Typically, autoinduced expression produces a greater proportion of soluble target protein than does IPTG-induced expression. Overnight Express™ Autoinduction Systems use optimized media components to promote culture growth to high cell densities, followed by lactose-induced protein expression from *lac*-based promoters. This method provides higher protein yields and greater convenience compared to standard IPTG induction.

### What specific media components are used in the Overnight Express Autoinduction Systems?

The Overnight Express Autoinduction Systems use an optimized blend of carbon sources, including lactose. The carbon sources are metabolized differentially to initially promote tightly regulated, uninduced growth, followed by autoinduction with lactose and continued cell growth, which results in high cell density at harvest. OnEx™ Solution 1 provides the blend of carbon sources.

To grow bacterial cultures to high cell densities, metabolic acids must be buffered to maintain pH near neutral. For increased protein expression, additional nitrogen is also necessary. OnEx Solution 2 provides both a concentrated buffer and an additional nitrogen source, so that cultures attain high cell densities and increased protein expression, even when grown in shake flasks or 96-well plates.

Magnesium is essential for cell growth, and higher concentrations of magnesium have been shown to increase the viability of cultures stored at 4°C for several weeks (1). OnEx Solution 3 provides the critical magnesium necessary to attain maximum cell density and improved culture viability.

### What are the differences between the three Overnight Express products?

The Overnight Express Autoinduction System 1 (2) includes OnEx Solutions 1-3, which are ready to be added to any glucose-free complex medium, such as Luria-Bertani (LB) broth or Terrific Broth (TB).

The Overnight Express Autoinduction System 2 includes OnEx Solutions 1-3 and OnEx Solutions 4-6, which together comprise a complete, defined autoinduction medium when added to water. Using the System 2 defined autoinduction medium, target proteins can be efficiently labeled with selenomethionine (Se-Met).

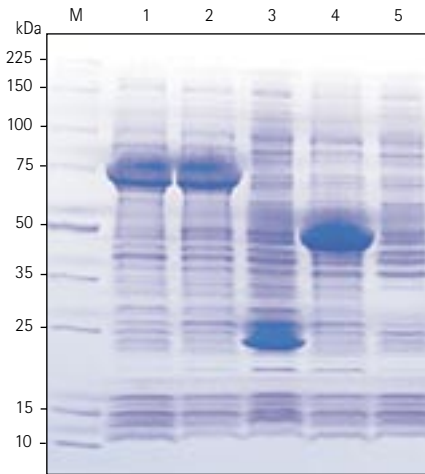
The Overnight Express Instant TB Medium is a complete, granulated, autoinduction medium, which includes the components found in the OnEx Solutions 1-3. Simply add the granules to water, supplement with glycerol, microwave or autoclave to sterilize, and inoculate. (For more information, please see p. 3.)

### What bacterial protein expression systems have been tested with the Overnight Express Autoinduction Systems?

Overnight Express Systems have been tested primarily with the pET protein expression system, particularly with T7lac promoter vectors. However, experiments with other IPTG-inducible promoters (i.e., *tac* and *trc* promoters) have also been successful (Figure 1). Based on the overall mechanism of the system, Overnight Express should be compatible with any IPTG-inducible promoter used for protein expression.

### Which expression hosts are compatible with the Overnight Express Autoinduction Systems?

Because lactose is used for induction, expression hosts producing functional Lac permease (encoded by the *lacY* gene) and β-galactosidase (encoded by the *lacZ* gene) are required. Strains with nonfunctional Lac permease will



**Figure 1. Comparison of T7 and other IPTG-inducible promoters**

Plasmids were transformed into BL21(DE3) (pET43.1) or BL21 (non-T7 promoters), and grown at 37°C in 30 ml LB + carbenicillin (lane 1) or LB + carbenicillin plus OnEx™ Solutions 1, 2, and 3 (lanes 2, 3, 4, and 5). The culture in lane 1 was induced with 1 mM IPTG at an OD<sub>600</sub> of 0.6 and harvested after 3 h. Other cultures were autoinduced overnight (16 h). M: Perfect Protein™ Markers, 10–225; 1: pET43.1 (T7/*lac*, 66.1 kDa) IPTG; 2: pET43.1 (T7/*lac*, 66.1 kDa); 3: pGEX (*tac*, 24.5 kDa); 4: pMal (*tac*, 43 kDa); 5: pTrc (*trc*, 37 kDa; the insert in pTrc is vTPA, which contains 32 rare codons).

not efficiently transport lactose for induction, and strains with nonfunctional  $\beta$ -galactosidase will not convert a portion of the transported lactose to the necessary allolactose inducer. The system is compatible with host strains such as BL21, Rosetta™, Rosetta 2, and their DE3 lysogen derivatives.

If the expression vector uses a *T7lac* promoter, as many of the pET vectors do, a host strain without a pLysS plasmid is recommended. The combination of the T7 lysozyme (expressed by the pLysS plasmid) and the *lac* repressor (encoded by vectors carrying the *T7lac* promoter) causes significantly reduced protein expression in the Overnight Express Autoinduction Systems. When the “plain” T7 promoter is used, the low level of lysozyme produced by pLysS has little effect on expression of target proteins.

## How long are cultures grown in the Overnight Express System?

With the Overnight Express Systems, cultures need to be grown to stationary phase. During IPTG induction, all of the cell’s energy is devoted to producing target protein. In contrast, the Overnight Express Systems induce target protein expression while the cells continue to grow. Autoinduction leads to cultures with higher cell densities and subsequently more soluble protein.

When using the cell culture guidelines outlined in the User Protocol (3) and growing the cells at 37°C, stationary phase is usually reached quickly, about 8–10 hours. If uncertain whether cultures have reached saturation, we recommend measuring OD<sub>600</sub> and continuing to grow the cells until the OD<sub>600</sub> plateaus. Incubation continued for several hours after stationary phase seems to have no deleterious effects.

Growth and induction at 25°C or 30°C may be optimal to improve the yield of soluble protein or to export target protein using signal sequence leaders present in a number of pET vectors. With lower incubation temperatures, saturation may require incubation for 24 hours or more. When using lower temperatures it may be helpful to initially incubate the culture at 37°C for 2–2.5 hours, reduce the temperature to the desired growth temperature, and grow the culture until it reaches stationary phase (at least overnight).

## Does Overnight Express work in fermentation systems?

Yes, Overnight Express was tested in a 15-L fermentation system and produced higher cell densities and better protein yields per liter than the IPTG-induced system to which it was compared. For additional information and data, please see the article on p. 3.

## Can I use Overnight Express to express proteins that are toxic to *E. coli*?

Although results will vary for different proteins, generally the Overnight Express Systems are suitable for toxic proteins. Because basal expression is tightly regulated, Overnight Express could improve the expression of some toxic proteins. Proteins known to be highly toxic to some *E. coli* strains, such as T7 gene 5.3 and 7.7 proteins (1), have been successfully expressed using Overnight Express.

### REFERENCES

1. Studier, F. W. (2005) *Protein Express. Purif.* 41, 207–234.
2. Grabski, A., Mehler, M., and Drott, D. (2003) *inNovations* 17, 3–6.
3. Novagen User Protocol TB383.