The Overnight Express Autoinduction System: High-density cell growth and protein expression while you sleep

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The Overnight Express Autoinduction Systems enable regulated protein expression in *Escherichia coli* without the need to monitor the culture or add inducer during cell growth and are marketed exclusively through the Novagen brand of EMD Biosciences, Inc. (EMD Biosciences). EMD Biosciences is a major supplier of premium tools for proteomics and disease pathway analysis known through its global brands Calbiochem, Novabiochem and Novagen and is part of the Life Science and Analytics (LSA) division of Merck KGaA (Darmstadt, Germany). The company operates as EMD Biosciences, Inc. in North America and as Merck Biosciences outside North America.

Recent developments in molecular biology have greatly streamlined the process of protein expression. One such development is the autoinduction system, which is designed for high-level protein expression with pET[1] and other isopropyl β-D-thiogalactopyranoside (IPTG)-inducible bacterial expression systems[2] without the need to monitor cell growth. F.W. Studier was recognized through an R&D 100 Award for his research that has been commercialized as the Overnight Express Autoinduction Systems (http://www.bnl.gov/bnlweb/pubaf/pr/PR_display.asp?prID=04-77). These systems often increase cell mass and target-protein yield substantially compared with conventional recombinant *E. coli* cell culture and induction with IPTG[1]. This method of growth and induction relies on medium components that are metabolized differentially to promote high-density growth and automatic induction of protein expression from *lac*-based promoters. Overnight Express provides a convenient approach for routine expression of proteins in multiple cultures in either complex (System 1 and Instant TB Medium) or defined (System 2) media and are ideally suited for high-throughput parallel analysis of protein expression and solubility as well as for purification from multiple expression clones. Further, System 2 can be used for selenomethionyl (SeMet) labeling of proteins for crystallography studies.

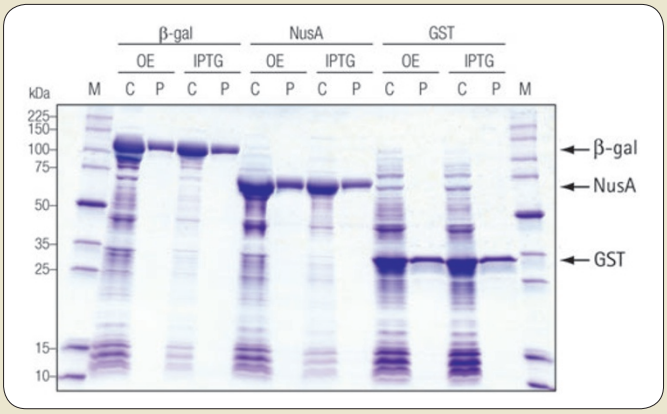

Figure 1 | SDS-PAGE analysis of crude and purified proteins from cultures autoinduced with Overnight Express System 1 or induced by IPTG. Proteins expressed by autoinduction or IPTG induction show similar purity, but the autoinduced cultures yielded more of the target protein as a proportion of the total protein than did the IPTG-induced cultures. OE is Overnight Express autoinduction, and IPTG is IPTG induction. M, Perfect Protein Markers (10–225 kDa); C, crude protein extract (equal volumes loaded); P, purified target protein (equal protein mass loaded).

Overnight Express System 1

The Overnight Express System 1 (Cat. No. 71300) contains three solutions. OnEx Solution 1 is a blend of carbon sources optimized for tightly regulated uninduced growth to high cell density followed by induction with lactose and continued growth. OnEx Solution 2 is a concentrated buffer that maintains pH throughout metabolic acid production and supplies additional nitrogen necessary to support increased protein synthesis. OnEx Solution 3 provides critical magnesium for maximum cell density. Addition of these components to traditional glucose-free complex media for growing cultures of *E. coli*, such as Luria-Bertani (LB) broth, Terrific Broth[3] (TB) or animal-free medium (Veggie medium; EMD Biosciences), results in high cell densities, autoinduction and maximum yields of target proteins with pET[1] or other IPTG-inducible systems. But the levels of induction may vary depending on medium composition,

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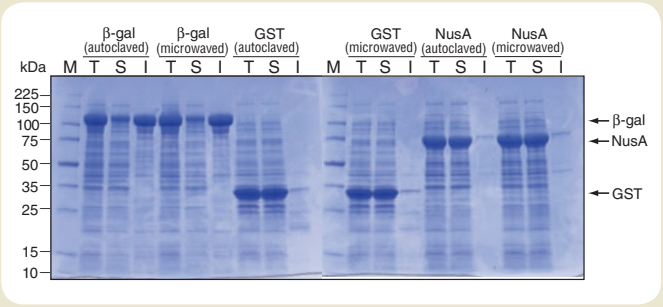

Figure 2 | Comparison of protein expression from Overnight Express Instant TB Medium prepared by autoclave or microwave. Overnight Express Instant TB Medium was either autoclaved to sterilize or microwaved before inoculation with cells that contain the expression vector encoding β-gal, GST or NusA. The average cell harvest OD_{600} was similar for each of the paired cultures. M, Perfect Protein Markers (10–225 kDa); T, total cell protein; S, soluble cell protein; I, insoluble cell protein.

cell growth stage and nutrient availability[4]. Because lactose is used for induction, the expression hosts must produce functional lactose permease (encoded by the *lacY* gene) and β-galactosidase (β-gal, encoded by the *lacZ* gene) for consistent results in both complex and defined media.

The System 1 kits provide sufficient reagents to supplement either 1 or 5 l of media. We tested Overnight Express autoinduction using three media and three vectors. The media were ZY, consisting of 10 g/l tryptone and 5 g/l yeast extract; TB, consisting of 13.3 g/l tryptone, 26.7 g/l yeast extract and 4.4 ml/l glycerol; and TYMV, consisting of 20 g/l Veggie Peptone, 5 g/l Veggie yeast extract and 100 mM NaCl. The pET vectors we used were pET-41b(+) to express a 35.6-kDa GST•Tag/His•Tag/S•Tag fusion protein (GST); pET-43.1b(+) to express a 66.4-kDa NusA•Tag/His•Tag/S•Tag/HSV•Tag fusion protein (NusA); and pET-30b(+) to express a 121-kDa His•Tag/S•Tag β-galactosidase fusion protein (β-gal). Each plasmid was separately transformed into the BL21(DE3) expression host. We chose these targets to test the capabilities of autoinduction with soluble (NusA), partially soluble (GST) and insoluble (β-gal) proteins. To compare cell growth, protein expression level and final purified target yield, we also performed a standard induction with IPTG using the same vectors, host and fusion proteins.

Autoinduction was accomplished by inoculating a single colony into 2 ml of ZY medium in 17 × 100-mm, round-bottom, snap-cap tubes and incubating overnight (approximately 16 h) at 30 °C with shaking at 300 r.p.m. IPTG induction was accomplished by inoculation of a single colony into 2 ml of ZY medium with 0.5% glucose in 17 × 100-mm, round-bottom, snap-cap tubes and incubation at 30 °C at 300 rpm until the culture reached an average OD_{600} of 2.2, followed by addition of IPTG to 1 mM final concentration and incubation for an additional 5 h before harvest. Medium for the IPTG-induced cultures included OnEx Solution 3 to eliminate $MgSO_4$ as a variable and demonstrate the influences of only OnEx Solution 1 and OnEx Solution 2 on cell harvest density (OD_{600}) and pure protein yield. However, cells cultured in ZY medium lacking OnEx Solution 3 and induced with IPTG may not achieve similar cell densities.

The crude soluble extract and purified fusion proteins were analyzed by SDS-PAGE. A protein gel of cultures grown in ZY medium shows that use of the Overnight Express System 1 resulted in the production of larger amounts of total protein for a given culture volume than were produced by IPTG-induced cells (**Fig. 1**). SDS-PAGE analysis of cultures grown in TB and TYMV medium yielded similar results. The purified samples (2 μg loaded) from the IPTG-induced and autoinduced cultures were of equivalent purity. These results correlate with the final cell density as well as with the purified target protein yields. The largest difference appeared in cell cultures using TB medium. Here, the autoinduced cultures yielded nearly three times as much purified protein as did the IPTG-induced cultures (data not shown). Autoinduction in ZY and TYMV media, respectively, yielded about twice as much purified β-gal protein, between 1.6 and 1.7 times as much NusA, but 1.3 and 0.9 times as much GST as did IPTG induction (data not shown). Cells cultured and autoinduced in System 1 had an average harvest OD_{600} of 17.3 compared to 10.5 for those induced with IPTG; purified protein yields averaged 510 and 253 μg/ml culture, respectively (data not shown).

Overnight Express Instant TB Medium

Overnight Express Instant TB Medium (Cat. No. 71491) is a complete granulated culture medium based on System 1 and TB medium and is supplied in two formats. EasyPak is an aluminum foil pouch containing 60 g granulated medium sufficient for a 1-l culture. Just add the EasyPak contents to 1 l of water, supplement with 10 ml glycerol, and either autoclave to sterilize or microwave for a few minutes. In addition, the Overnight Express Instant TB Medium is supplied in 1-kg bottles. The granules ensure rapid and uniform dissolution in water, preventing clumping of the medium and inhalation of airborne powder.

We compared the results of autoclaving and microwaving prepared Instant TB Medium using three different pET system recombinants. In these experiments, Overnight Express Instant TB Medium was prepared according to the user protocol and either autoclaved to sterilize or microwaved. After cooling the media to 37 °C, antibiotics were added, and the media were inoculated separately with the

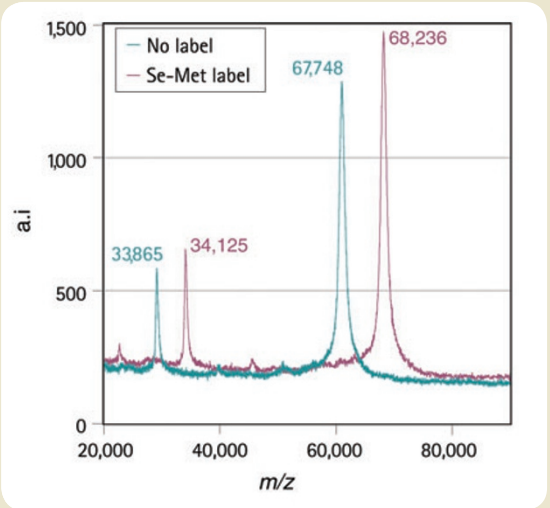

Figure 3 | Mass spectrometry analysis showing Se-Met incorporation in cells grown with Overnight Express System 2. Cells expressing the target protein were grown in System 2 medium containing either Se-Met or Met. MS analysis demonstrates that the target protein produced with System 2 autoinduction incorporated Se-Met. a.i., ionization signal absolute intensity. (Spectra provided by the Mass Spectrometry/Bioanalytical Facility at the University of Wisconsin Biotechnology Center.)

expression clones. Cultures of *E. coli* expression host BL21(DE3) carrying pET-30b(+) to express β-gal, pET-41b(+) to express GST or pET-43.1b(+) to express NusA were grown for approximately 16 h at 37 °C with shaking at 300 r.p.m. in 17 × 100-mm snap-cap tubes containing 2.5 ml Overnight Express Instant TB Medium. Cultures (2 ml) were harvested by centrifugation (9,000*g* for 5 min) and treated with 500 µl BugBuster Protein Extraction Reagent + 1.0 µl Lysonase Bioprocessing Reagent (Lysonase) to lyse the cells and extract cell proteins. Equivalent amounts of fractionated cellular extracts were analyzed by SDS-PAGE (10–20% gradient gel) and Coomassie blue staining (**Fig. 2**). Sample load amounts were normalized according to the cell harvest OD_{600}. The average cell harvest OD_{600} was very similar for each of the paired cultures (**Table 1**).

Overnight Express System 2

The Overnight Express System 2 (Cat. No. 71366) contains OnEx Solutions 1–3 plus three additional components, OnEx Solutions 4–6. OnEx Solution 4 provides trace metals below toxic levels to minimize growth limitations associated with mineral deficiencies; it saturates almost any metal-containing target protein even at high expression levels. OnEx Solution 5 is an amino acid mixture lacking methionine, cysteine and tyrosine. OnEx Solution 6 is an individual solution of methionine (Met). Addition of these six components to sterile water results in a defined medium capable of promoting high cell densities, enabling autoinduction of expression, producing maximum soluble protein yields and, if desired, efficient labeling of target proteins through the addition of Se-Met[5].

The System 2 kits provide sufficient reagents for 1 or 5 l of media. We used mass spectrometry (MS) analysis to measure Met and Se-Met incorporation in a pET-encoded recombinant protein. In this experiment, B834(DE3) cells containing the pET-44b(+) to express a 67.8-kDa His•Tag/Nus•Tag/His•Tag/S•Tag/HSV•Tag fusion protein (His•Tag) were grown for 16 h at 37 °C in 500-ml baffled flasks in OnEx System 2 Medium containing either Se-Met (125 µg/ml) or Met (OnEx Solution 6). Cells were collected by centrifugation and resuspended in Ni-NTA Bind Buffer containing 4-(2-aminoethyl)benzenesulfonylfluoride•HCl (AEBSF hydrochloride), benzamidine hydrochloride and Lysonase. The suspension was sonicated and centrifuged at 12,000*g* for 10 min. The supernatant (soluble protein) was processed by Ni-NTA His•Bind chromatography. Two micrograms of purified protein, 5 µl of soluble cell protein and a sample of crude extract (standardized to cell harvest OD_{600}) were analyzed by SDS-PAGE (10–20% gradient gel) and Coomassie blue staining. The protein was assayed using the BCA Protein Assay Kit (EMD Biosciences).

When the purified protein was subjected to MS as shown in **Figure 3**, the analysis showed Se-Met incorporation into the target protein with System 2 autoinduction.

Table 1 | Comparison of microwave heating and autoclave sterilization for preparation of Overnight Express Instant TB Medium

Recombinant protein	Preparation method	Average cell harvest OD_{600} (n = 3)
β-gal	Autoclave	23.1
β-gal	Microwave	29.7
GST	Autoclave	23.8
GST	Microwave	23.0
NusA	Autoclave	26.0
NusA	Microwave	22.5

Conclusions

We compared the Overnight Express Autoinduction System 1 with standard IPTG induction using three different pET recombinants and three different medium formulations. Using cultures in 2-ml tubes, three different recombinants averaged 1.7-fold higher OD_{600} at cell harvest and twofold higher yield of purified protein with the Overnight Express system. In addition, we verified that Overnight Express Instant TB Medium produces similar yields, whether the medium is autoclaved or microwaved. Finally, we demonstrated that using System 2 with Se-Met enables production of protein that has incorporated Se-Met, and is thus suitable for X-ray crystallography studies. In addition to their superior performance, the Overnight Express systems are extremely convenient because they allow growth and induction of recombinant proteins without the need to monitor cell density or add inducer. These features can greatly streamline many protein expression applications, from parallel analysis of multiple small-scale cultures to larger scale processing for preparative uses[a].

[a]The products and technologies presented in this article were developed and are offered under the terms of a license agreement between EMD Biosciences, Inc., Novagen Brand and Brookhaven Science Associates, covering US patent properties entitled "High Density Growth of T7 Expression Strains with Auto-Induction Option," filed March 14, 2003, in the name of F. William Studier and assigned to Brookhaven Science Associates, LLC, Upton, New York 11973, USA. The Autoinduction Media Technology embodied in the Overnight Express Autoinduction Systems is based on technology developed at Brookhaven National Laboratory under contract with the US Department of Energy and is the subject of patent applications assigned to Brookhaven Science Associates, LLC. This product is to be used for research purposes only. A separate license is required for any commercial manufacture or use, including the manufacture of protein products for use in the screening of compound libraries. Information about commercial licenses may be obtained from the Office of Intellectual Property and Industrial Partnerships, Brookhaven National Laboratory, Bldg. 475D, P.O. Box 5000, Upton, New York 11973-5000, USA; telephone (631) 344-7134.

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