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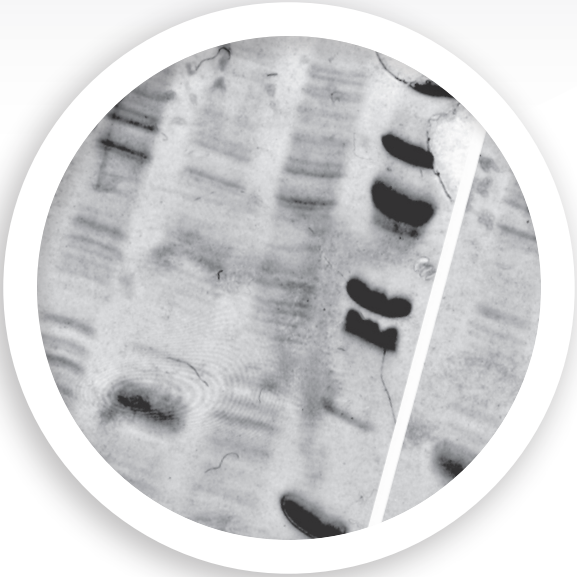
APPLICATION NOTE 12

Optimization of BioExpress® Supplementation of M9 Cultures

Marwa Rhima, Lori C. Neil and Kevin H. Gardner
Department of Biochemistry, UT Southwestern Medical Center at Dallas, Dallas, TX 75235 USA



Uniform labeling of proteins with ^{15}N and ^{13}C has typically been achieved through the use of bacteria grown in minimal media, such as M9, which contain single nitrogen and carbon sources. While this arrangement facilitates the straightforward isotopic replacements of these elements, the growth characteristics of *E. coli* in these media are somewhat compromised compared to growth in rich media. These effects typically include a drop in maximum cell density, requiring that larger cultures be grown to produce sufficient quantities of protein for NMR study.

To avoid the increased expense and time required to prepare, grow and process such larger cultures, an alternative approach is to supplement minimal media with mixtures of isotopically labeled biomolecules, such as cell lysates. Here we evaluate the effects of adding increasing amounts of one such mixture, BioExpress® Cell Growth media from Cambridge Isotope Laboratories, Inc., to *E. coli* cultures grown in M9 minimal media. We characterize the beneficial effects of this supplementation on cell growth rates, maximal densities and protein expression and observe significant benefits in all three of these categories.

Methods

Escherichia coli strain BL21(DE3) (Stratagene) was transformed with the plasmid Gβ1-STOP, encoding the streptococcal protein Gβ1 domain (Gβ1) under control of a T7 RNA polymerase promoter, and plated on an LB/amp plate. A colony from this plate was inoculated into 5 mL of LB media and allowed to grow for 4 hr at 37°C with vigorous shaking. At this point, the culture was divided into equal parts, centrifuged, and pellets resuspended in M9 media (Table 1) supplemented with various quantities of BioExpress® Cell Growth media. For an initial test of growth characteristics without protein induction, these media contained BioExpress® at concentrations of 0.5%, 1%, 10%, 20% and a standard M9-only sample. A subsequent test of growth characteristics with protein induction used cultures containing 0.5%, 1%, 2%, 5%, 10% and a standard M9-only sample. For this latter set, protein expression was induced by the addition of 0.5 mM IPTG at a point during the middle of log phase growth (typical A_{600} values ~ 0.4-1.2) as determined from the prior test without induction. At all points through these growths,

(continued)

cultures were grown at 37°C with vigorous shaking, and densities were monitored by turbidity at 600 nm (A_{600}). Induced cultures were harvested three hour post-induction, at which point they were analyzed for protein expression (SDS-PAGE) and total cell mass (wet weight).

Table 1: M9 Minimal Media

Na_2HPO_4	6 g/L	$MgSO_4 \bullet 7H_2O$	246 mg/L (1 mM)
KH_2PO_4	3 g/L	$CaCl_2 \bullet 2H_2O$	14.7 mg/L (0.1 mM)
NaCl	0.5 g/L	thiamine	10 mg/L
NH_4Cl	1 g/L	biotin	10 mg/L
glucose	3 g/L	ampicillin	100 mg/L

Results

Supplementing standard M9 media with BioExpress® Cell Growth media provided several significant benefits, including decreased doubling times and increased total cell mass. These favorable characteristics were observed in cultures supplemented with BioExpress® at amounts significantly below the recommended working level of 1% v/v. In detail:

- Supplementing M9 media with BioExpress® significantly shortened doubling times by up to 40%. As detailed in Table 2, BioExpress®-supplemented M9 cultures had doubling times between 31-49 minutes, as compared to 57 minutes for a non-supplemented culture. The degree of doubling time shortening correlated with the amount of added BioExpress®, with the most pronounced effects observed at concentrations of at least 1%.

Effect of BioExpress® supplementation on doubling times, cell densities

media	doubling time (min)	max A600 (non-induced)	A600 (3 hr post induction)
Control (M9)	57.3 min	2.20	0.92
M9 + 0.5% v/v BioExpress®	48.7 min	2.86	1.75
M9 + 1%	38.2 min	3.09	1.63
M9 + 2%	34.7 min	n.d.	1.97
M9 + 5%	33.3 min	n.d.	1.90
M9 + 10%	31.6 min	5.26	2.81

- Adding BioExpress® significantly increased the maximum density obtained from these cultures, regardless of whether protein expression was induced or not. As shown in Table 2 and Figure 1, supplementation with BioExpress® at levels as low as 0.5% v/v increased post-induction densities by factors of two-threefold compared to M9 controls.

- BioExpress®-supplemented cultures also expressed higher amounts of the induced G1 protein on a per-cell basis, as shown in the SDS-PAGE analyses presented in Figure 2. Importantly, this effect is specific to the induced protein and does not represent an increase in the levels of all proteins, as shown by the equivalent staining of non-induced proteins across all of the samples in Figure 2.

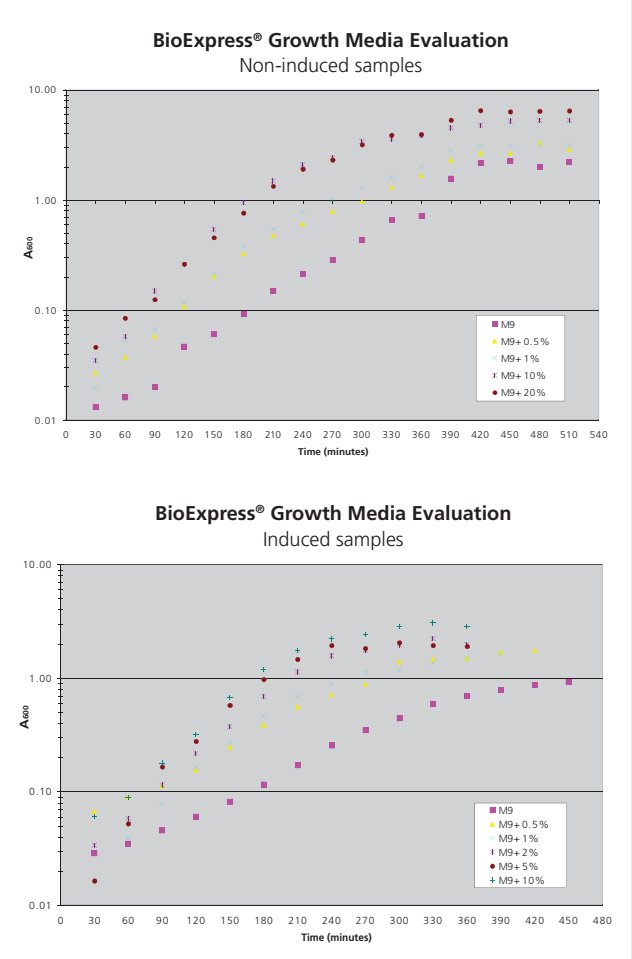


Figure 1. Growth curves of BL21(DE3) cells containing pGβ1-STOP in M9 media supplemented by various concentrations of BioExpress® Cell Growth Media. Protein expression in the induced cultures was triggered by the addition of 0.5 mM IPTG at mid-log phase points that ranged from A600~0.4 (M9) to A600~1.2 (M9+10% BioExpress®).

Summary

The supplementation of standard M9 media with complex mixtures of biomolecules and their precursors, such as BioExpress® Cell Growth media, offers a route to straightforwardly improve the yield of uniformly labeled protein from bacterial cultures. As shown here, this supplementation can improve three key aspects of this process: growth rate, maximal cell density, and the degree of overexpression of a recombinant protein. Significant improvements in each of these can be obtained by supplementing with modest (1% v/v) amounts of BioExpress®, with further improvement possible at higher levels.

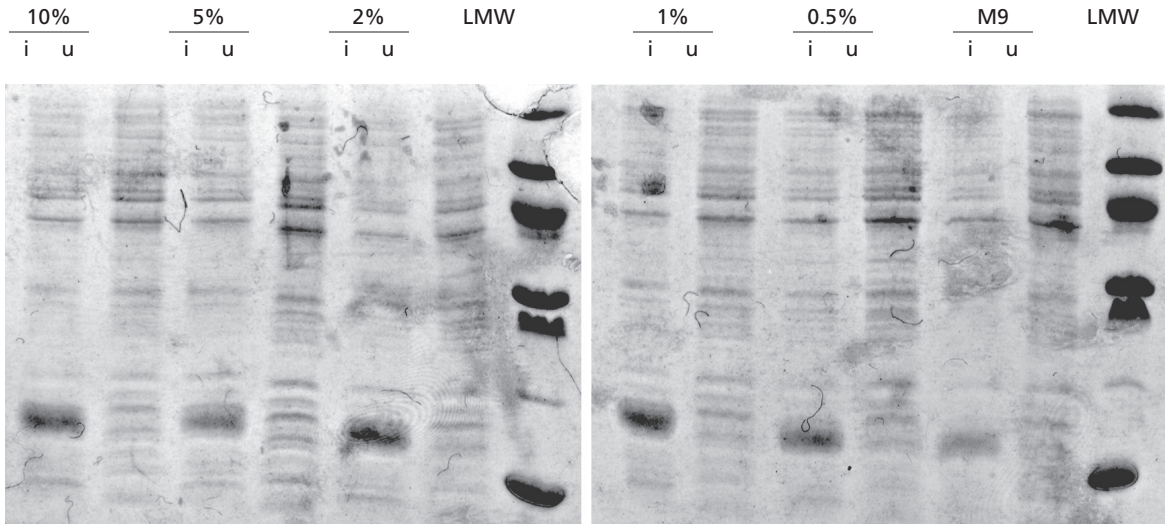


Figure 1. SDS-PAGE analysis of whole cells from non-induced and induced cultures of cells grown in M9 media containing varying concentrations of BioExpress® Cell Growth media. Both uninduced (u) and induced (i) samples of cultures grown with each concentration of BioExpress® were evaluated as identified by the percentage supplementation of BioExpress®. All samples were corrected for cell density prior to loading. The band corresponding to Gβ1 is identified with an arrow on the left side of the figure. LMW: low molecular weight markers.

Cambridge Isotope Laboratories, Inc., 3 Highwood Drive, Tewksbury, MA 01876 USA
tel: +1.978.749.8000 | fax: +1.978.749.2768 | 1.800.322.1174 (North America) | www.isotope.com