Using ISOGRO® Supplementation of M9 Minimal Media to Enhance Recombinant Protein Expression

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Stable isotope enrichment of recombinant proteins is necessary for most structure-function studies using NMR spectroscopy. The uniform incorporation of stable isotopes such as 13C, 15N and D into proteins is routinely accomplished by using bacterial expression systems such as Escherichia coli (E. coli). Protein expression in E. coli typically involves the growth of bacteria in defined media where 13C and 15N containing glucose and ammonium salt are the sole sources of carbon and nitrogen. While bacterial protein production systems offer high cell mass and protein yields in rich media, the level of protein expression and cell mass obtainable in defined media is often lower. To compensate for reduced cell mass and recombinant protein yields, the large-scale production of protein in E. coli cells grown in M9 minimal or complex media is required which can be labor-intensive and expensive. Economical and efficacious production of adequate quantities of isotopically enriched protein for NMR experimentation is often the rate-limiting step in structural biology. Therefore, considerable interest exists in the development of protocols for the improvement of growth rates and recombinant protein expression levels in E. coli.

One strategy for the cost-effective optimization of protein expression in E. coli is the supplementation of M9 media with ISOGRO, an algal lysate-derived complex labeling medium. The inclusion of ISOGRO in M9-based growth cultures provides the cells a metabolic boost that often decreases lag time, facilitates the attainment of growth saturation, and promotes recombinant protein production. The presence of algal cell lysate-derived complex growth media in cultures conserves cellular energy by limiting the requirement for de novo synthesis of cellular machinery and metabolic precursors. Minimizing energy expenditure permits more cellular resources to be directed towards recombinant protein expression. This protocol describes the usage of ISOGRO as a supplement in minimal media to augment E. coli cell growth and protein production.

Advantages of Supplementation

- Cost-effective protein production in E. coli with as little as 10% ISOGRO to M9
- Decrease lag time by as much as 60%
- Maximize OD and recombinant protein expression
- High level of recombinant protein expression with ISOGRO supplementation

Method

Competent E. coli BL21(DE3) pLysS cells were freshly transformed with the expression plasmid containing the gene encoding for troponin C amino acid residues 1-89 (cTnC1-89) controlled by the T7 promoter, spread aseptically on LB plates containing carbenicillin (Carb) and chloramphenical (Chl), and grown overnight at 37 °C. A single colony was grown at 37 °C in LB with antibiotic selection until slightly turbid with shaking at 250 rpm. From this starter culture, 1 mL was transferred to 50 mL of LB/Carb/Chl, grown at 37 °C with shaking, and the optical density was monitored at 600 nm (OD600). The cells were harvested at OD600 ~ 0.9 by centrifugation and
resuspended in 50 mL of sterile filtered minimal media (pH 7.0) containing 7 g/L Na₂HPO₄, 3 g/L KH₂PO₄, 2.5 g/L NaCl, 10.5 g/L K₂HPO₄, 0.5 g NaOH, 1 g/L ¹⁵NH₄Cl, 4 mM MgSO₄, 10 µM FeCl₃, 125 µM CaCl₂, 50 µM ZnSO₄, 2 g/L D-Glucose-¹³C₆, 107 µg/L MgCl₂ ° 6 H₂O, 20 µg/L FeCl₂ ° 4H₂O, 0.16 µg/L MnCl₂ ° 4H₂O, 16 ng/L CuCl₂ ° 2H₂O, 2.4 ng/L Na₂MoO₄ ° 2H₂O, 0.26 µg/L H₃BO₃, 1 mg/L choline chloride, 100 µg/L riboflavin, 50 mg/L niacin, 1 mg/L folic acid, 1 mg/L pyridoxal phosphate, 50 mg/L thiamine, 1 mg/L biotin, 1 mg/L D-pantothenate. The minimal media starter culture was equally divided between 500 mL regular minimal medium and 500 mL of minimal media supplemented with 10% ISOGRO 13C,15N powdered growth medium (0.5 g ISOGRO powder/500 mL medium) and grown at 37 °C with shaking. At an OD₆₀₀ ~ 0.9 the protein expression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM. Protein production was analyzed by SDS-PAGE.

Results

Bacterial Growth Rates

Supplementation of minimal media with 10% ISOGRO substantially reduced the lag time of cells by more than 60% as evidenced by the faster transition of cultures into log phase growth (see Figure 1). Cells grown in ISOGRO supplemented minimal media reached the target induction point of an OD₆₀₀ ~ 0.9 in approximately 3 hours, versus 9 hours in standard M9 media. ISOGRO is composed of an algal lysate containing metabolic precursors, which permits the cultures to initiate growth more rapidly, thus eliminating the need for de novo production of the various components of the metabolic pathways.

![Figure 1: Growth Curve cTnC(1-89) p LysS. OD₆₀₀ versus time (hours). Red curve is ISOGRO supplemented minimal media and the black curve is minimal media alone.](image)

Recombinant Protein Expression

The expression levels observed for cTnC(1-89) produced in the ISOGRO enriched minimal media were greater compared to that obtained using unsupplemented minimal media (see Figure 2). In the presence of 10% ISOGRO, there was an increase in the amount of cTnC(1-89) expressed while the contaminating host cellular proteins were produced at a level comparable to that seen in uninduced minimal media and induced minimal media cultures. This observation is important in
that subsequent recombinant protein purification is often facilitated when the target protein constitutes a high percentage of the total cellular protein.

![SDS-PAGE cTnC(1-89) cell lysates. M = low molecular weight marker, 0 = uninduced ; 1 = induced minimal media ; 2 = induced minimal media + ISOGRO, L = Lysozyme](image)

**Figure 2:** SDS-PAGE cTnC(1-89) cell lysates. M = low molecular weight marker, 0 = uninduced ; 1 = induced minimal media ; 2 = induced minimal media + ISOGRO, L = Lysozyme

**Discussion**

Modern NMR techniques require the routine production of milligram quantities of uniformly enriched recombinant protein. It is desirable to develop protocols for the economic and time-efficient isotopic enrichment of target proteins. The methodology presented here offers researchers the potential to reduce the labor hours and isotope expense by utilizing ISOGRO supplementation of minimal media for recombinant protein expression. The inclusion of 10% ISOGRO (0.5g/500 mL of minimal media) in minimal media reduces the lag time of cultures and promotes the enhancement of target protein levels. It should be noted that further optimization of this protocol is possible by altering variables such as IPTG concentration, induction optical density, and percentage of ISOGRO supplementation.

For more information on ISOGRO or other stable isotope labeled products, please visit sigma-aldrich.com/bionmr or contact Stable Isotopes Technical Service at isosales@sial.com

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