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Product Information

ISOGRO® Media

Catalog Numbers **606863, 616729, 606871, 606839, 608300, and 608297**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Stable isotope enrichment of proteins is necessary for study by NMR spectroscopy. Uniform isotope labeling is the biosynthetic labeling with stable isotopes (^{13}C, ^{15}N, and/or D) of all the respective sites in a protein. Routinely, this is accomplished using a bacterial (*E. coli*) expression system for recombinant proteins. The bacteria are grown in a defined, minimal medium with D-glucose-$^{13}C_6$ and ammonium-^{15}N salts as the sole sources of carbon and nitrogen, respectively. Bacterial growth under these conditions often results in reduced cell mass and low levels of recombinant protein expression (see Figure 2).

ISOGRO® media help overcome the growth limitations of minimal media. ISOGRO products are lysates of algae grown with stable isotopes (^{13}C, ^{15}N, and/or D). A typical algal lysate (ISOGRO medium) contains:

Salts	30%
Water	3%
Glucose	2%
Amino acids/peptides	65%

ISOGRO media may contain a single or multiple stable isotope(s).

Catalog Number	Growth Medium	Isotopic Purity
606863	ISOGRO-^{13}C Powder	99 atom % ^{13}C
616729	ISOGRO-D Powder	97 atom % D
606871	ISOGRO-^{15}N Powder	98 atom % ^{15}N
606839	ISOGRO-^{13}C, ^{15}N Powder	99 atom % ^{13}C 98 atom % ^{15}N
608300	ISOGRO-^{15}N, D Powder	98 atom % ^{15}N 97 atom % D
608297	ISOGRO-^{13}C, ^{15}N, D Powder	99 atom % ^{13}C 98 atom % ^{15}N 97 atom % D

ISOGRO products, whether used as stand-alone media or as supplements to minimal media, provide uniform labeling for protein expression NMR studies.

Stand-Alone Medium – ISOGRO media may be used as stand-alone media (see Procedures, section B). The following comparative results were observed:

Yield - Increased expression of recombinant protein compared to minimal medium (see Figure 3).

Growth rate (production time) – As a Quality Control measure, the suitability of each batch of ISOGRO media as a culture medium is determined by comparison to a LB Broth growth curve.

Supplement to Minimal Medium – ISOGRO media may be used to supplement minimal media (see Procedures, section A). Supplementation with complex ISOGRO media provides a metabolic boost to the cells by limiting the requirement for *de novo* synthesis of metabolic precursors. This results in more rapid growth and increased protein expression compared to minimal media alone:

Growth rate – as much as 60% decrease in lag time to log phase growth (see Figure 1).

Higher expression levels of recombinant protein – target protein is a higher percentage of total protein (see Figure 2).

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For long term storage, store the media at –20 °C.

Procedures

The following procedures are examples for use of ISOGRO media. They are presented as guidelines only. A researcher may have to modify the procedures depending on the transformed bacterial cells used, based on growth parameters, antibiotic selection, induction, etc.

A. ISOGRO Supplementation of Minimal Medium

1. Spread freshly transformed *E. coli* BL21(DE3) pLysS/(cTnC 1-89) cells on LB pate containing carbenicillin (Carb) and chloramphenicol (Chl), and grow overnight at 37 °C.
2. Inoculate a single colony in Luria Bertani Broth (LB) containing carbenicillin and chloramphenicol.
3. Grow at 37 °C with shaking at 250 rpm until slightly turbid.
4. Transfer 1 mL of starter culture to 50 mL of fresh LB/Carb/Chl medium and grow at 37 °C.
5. Harvest cells at OD_{600} ~0.9 by centrifugation.
6. Resuspend harvested cells (step 4) in 50 mL of sterile filtered Minimal Medium, pH 7.0, containing the following:

7 g/L Na_2HPO_4	1 mg/L D-pantothenate
3 g/L KH_2PO_4	1 mg/L biotin
2.5 g/L NaCl	50 mg/L thiamine
10.5 g/L K_2HPO_4	1 mg/L pyridoxal phosphate
0.5 g NaOH	50 mg/L niacin
1 g/L $^{15}NH_4Cl$	1 mg/L folic acid
4 mM $MgSO_4$	100 μg/L riboflavin
10 mM $FeCl_3$	1 mg/L choline chloride
125 mM $CaCl_2$	0.26 μg/L H_3BO_3
50 mM $ZnSO_4$	2.4 ng/L Na_2MoO_4•$2H_2O$
2 g/L D-Glucose-$^{13}C_6$	16 ng/L $CuCl_2$•$2H_2O$
107 μg/L $MgCl_2$•$6H_2O$	0.16 μg/L $MnCl_2$•$4H_2O$
20 μg/L $FeCl_2$•$4H_2O$	

7. Inoculate 50 mL of harvested cell suspension (step 5) into 1 L of Minimal Medium supplemented with 1 g/L of ISOGRO-^{13}C, ^{15}N Powder - growth medium (10% of amount recommended for use as a stand alone medium).
8. Grow at 37 °C with shaking at 250 rpm.
9. Induce cells at OD_{600} ~0.9 by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM.
10. Harvest cells eight hours post-induction.
11. Analyze protein production by SDS-PAGE.

B. ISOGRO Powder as a Stand-alone Medium (10 g/L)

1. Dissolve 0.5 g of appropriate ISOGRO powder into 45 mL of ultrapure water.
2. Make the following salt stock solutions and add the listed volumes to the preparation (step 1):

   0.9 mL of 100 g/L K_2HPO_4 solution
   1.4 mL of 50 g/L KH_2PO_4 solution
   1.0 mL of 50 g/L $MgSO_4$ solution
   15.0 μL of 37 g/L $CaCl_2$ solution

3. Adjust the pH to 7.0 with NaOH solution and bring the final volume to 50 mL with ultrapure water.
4. Add 2.5 mg of ampicillin to the solution.
5. In a positive flow hood, pass the mixture through a 0.2 μm filter and transfer the filtrate to a sterilized 500 mL flask. (Used 2 filters and 2 syringes).
6. Inoculate the culture with a loop of *E. coli*.
7. Shake at 37 °C at 200 rpm.
8. OD_{600} = 0.36 at 6.5 hours.
9. OD_{600} = 1.2 at 8 hours.
10. Save an aliquot for SDS-PAGE gel: 0.4 mL of 10-fold dilution from liquid used for OD_{600} measurement, spin down, and save pellet at –20 °C.
11. Change the shaker temperature to 20 °C, let the cells equilibrate for 30 minutes.
12. Induce cells using 25 μL of 1 M IPTG and incubate overnight at 20 °C at 225 rpm.
13. OD_{600} = 2.0 at 14 hours after induction.
14. Save an aliquot: 10-fold dilution from liquid used for OD_{600} measurement, spin down, and save pellet at –20 °C.

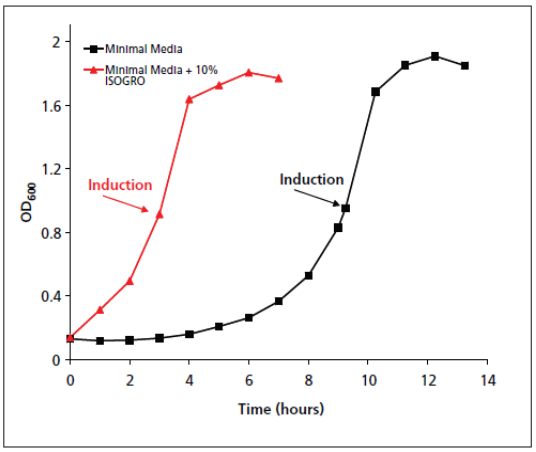
Results

A. ISOGRO Supplementation of Minimal Medium[1]

Cells grown on Minimal Medium with ISOGRO supplementation reached the induction point (OD_{600} ~0.9) in 3 hours compared to 9 hours with Minimal Medium alone (see Figure 1). ISOGRO supplementation reduced the lag time of cell growth by >60%.

Figure 1.

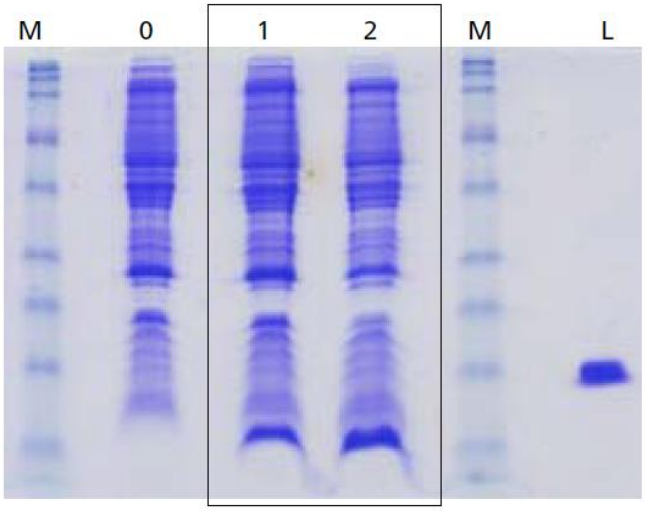
Growth Curve of *E. coli* BL21(DE3) pLysS/(cTnC 1-89) cells



Growth on Minimal Medium with ISOGRO supplementation resulted in increased amounts of the recombinant cTnC(1-89) protein, while host proteins were produced at levels comparable to those observed in Minimal Medium alone (see Figure 2).

Figure 2.

SDS-PAGE of cTnC(1-89) Cell Lysates

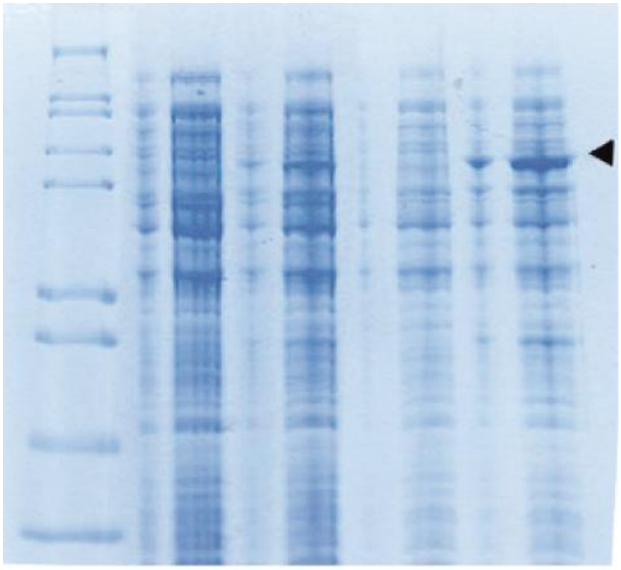


M – low molecular weight marker
0 – uninduced
1 – induced minimal medium
2 – induced minimal medium plus ISOGRO
L – Lysozyme

B. ISOGRO Powder as a Stand-alone Medium[2] (10 g/L)

A 39 μM sample of p38 alpha was produced from 50 mL of culture, see Figure 3.

Figure 3.
p38 alpha production



Lanes left to right:
Molecular weight marker
Uninduced minimal medium
Induced minimal medium
Uninduced ISOGRO medium
Induced ISOGRO medium
black arrow – p38 alpha

References

1. Data provided by Dr. Paul R Rosevear, Department of Molecular Genetic, Biochemistry, and Microbiology, University of Cincinnati Medical Center, Cincinnati, Ohio.
2. Data provided by Dr. Jeffery W. Peng, Department of Chem/ Biochemistry, University of Notre Dame, Notre Dame, Indiana.
3. Perez, C.L., and Van Gilst, M.R., A ^{13}C Isotope Labeling Strategy Reveals the Influence of Insulin Signaling on Lipogenesis in *C. elegans*. Cell Metabolism, **8**(3), 266-274 (2008).
4. Mullany, B.C. et al., Regulation of *C. elegans* Fat Uptake and Storage by Acyl-CoA Synthase-3 Is Dependent on NR5A Family Nuclear Hormone Receptor *nhr-25*, Cell Metabolism, **12**(4), 398-410 (2010).

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