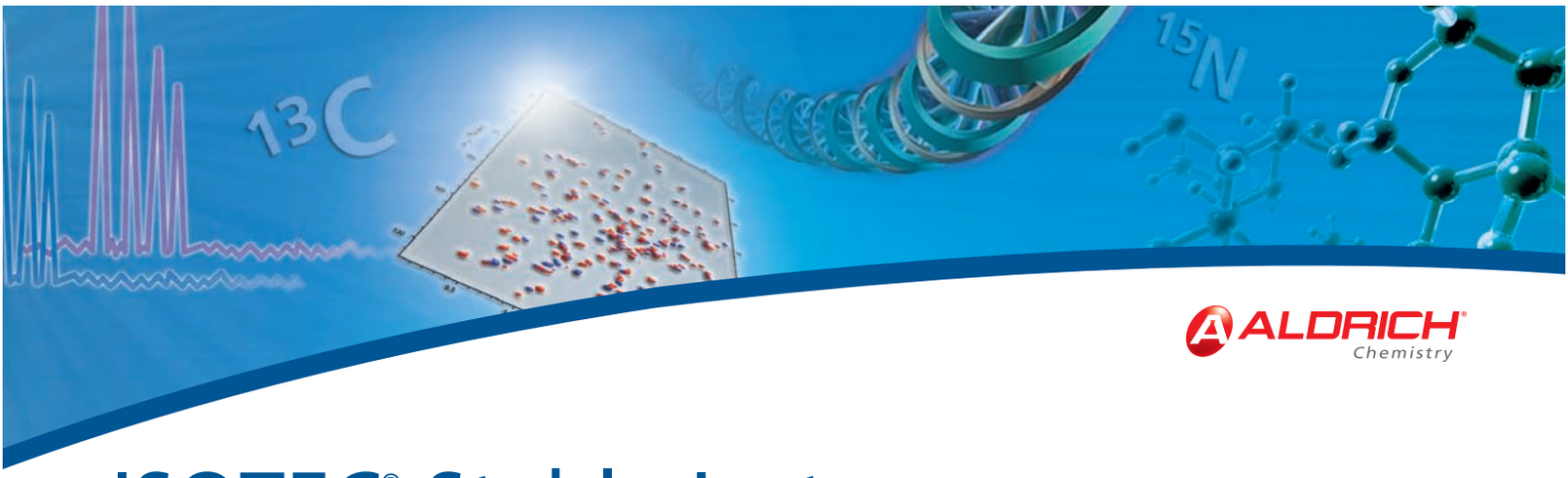


ALDRICH® Chemistry

ISOTEC® Stable Isotopes

Amplify Recombinant Protein Expression with ISOGRO®

- Decrease lag time by as much as 60%.
- Maximize OD and recombinant protein expression.
- Supplement with as little as 10% ISOGRO and improve the production of difficult to express proteins in *E. coli.*

Cat. No.	Description	Isotopic Purity
606863	ISOGRO-^{13}C Powder-Growth Medium	99 atom % ^{13}C
616729	ISOGRO-D Powder-Growth Medium	97 atom % D
606871	ISOGRO-^{15}N Powder-Growth Medium	98 atom % ^{15}N
606839	ISOGRO-^{13}C,^{15}N Powder-Growth Medium	99 atom % ^{13}C 98 atom % ^{15}N
608300	ISOGRO-^{15}N,D Powder-Growth Medium	98 atom % ^{15}N 97 atom % D
608297	ISOGRO-^{13}C,^{15}N,D Powder-Growth Medium	99 atom % ^{13}C 98 atom % ^{15}N 97 atom % D
299251	Ammonium -^{15}N chloride	98 atom % ^{15}N
299286	Ammonium -$^{15}N_2$ sulfate	98 atom % ^{15}N
617385	Deuterium oxide	99.8 atom % D
552003	D-Glucose -1,2,3,4,5,6,6-d_7	97 atom % D
389374	D-Glucose-$^{13}C_6$	99 atom % ^{13}C
552151	D-Glucose-$^{13}C_6$-1,2,3,4,5,6,6-d_7	99 atom % ^{13}C 97 atom % D

For more information on ISOGRO, related products, and procedural information visit *sigma-aldrich.com/bionmr*

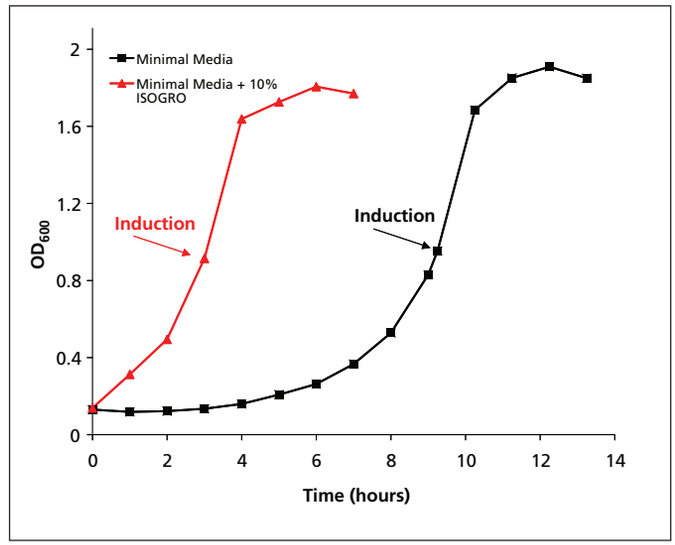


Figure 1. Cardiac troponin cTnC(1-89) in pLysS. Cells grown at 37 °C in shaker flasks. Red curve is ISOGRO supplemented minimal media and black curve is minimal media alone.

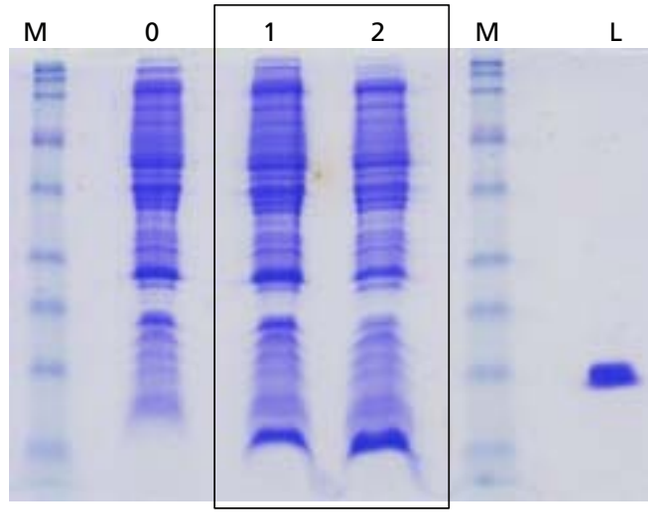
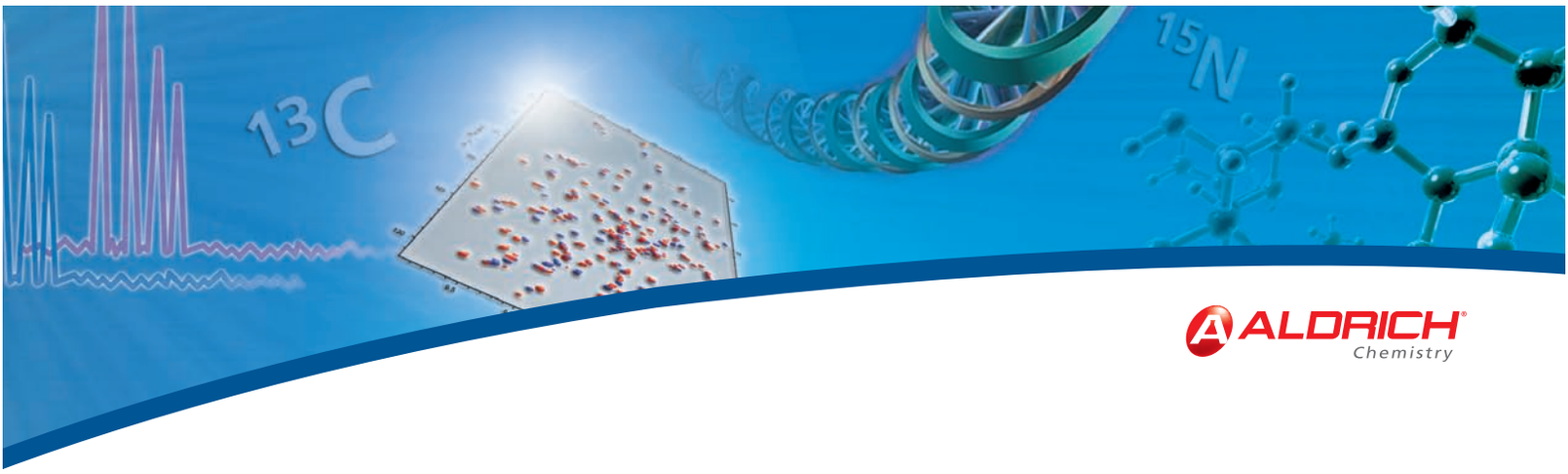


Figure 2. SDS-PAGE cTnC(1-89) cell lysates. M-low molecular weight marker; 0= uninduced; 1=induced minimal media; 2=induced minimal media + ISOGRO; L=Lysozyme. Data provided by Dr. Paul R. Rosevear The Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati Medical Center, Cincinnati, OH.



ALDRICH® Chemistry

Procedure for 10% ISOGRO Supplementation of M9 Minimal Media:

1. Inoculate E. coli BL21(DE3) pLysS/(cTnC 1-89) in Luria Bertani Broth (LB) containing Carbenicillin (Carb) and Chloramphenicol (Chl).
2. Grow at 37 °C with shaking at 250 rpm until slightly turbid.
3. Transfer 1mL to 50 mL of LB/Carb/Chl and grow at 37°C. Harvested cells at $OD_{600\ nm}$ ~ 0.9 by centrifugation.
4. Harvest cells at $OD_{600\ nm}$ ~ 0.9 by centrifugation.
5. Resuspend starter culture in 50 mL of sterile filtered minimal media (pH 7.0) supplemented with 1 g/L of ISOGRO®-^{13}C, ^{15}N powder growth medium and containing the following:

7 g/L $Na_2H\ PO_4$	1mg/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 $Na_2MoO_4 \bullet 2H_2O$
2 g/L D-Glucose $^{13}C_6$	16 ng/L $CuCl_2 \bullet 2H_2O$
107 µg/L $MgCl_2 \bullet 6H_2O$	0.16 µg/L $MnCl_2 \bullet 4H_2O$
20 µg/L $FeCl_2 \bullet 4H_2O$	

6. Inoculate 50 mL starter culture into 1 L of minimal media supplemented with 10% ISOGRO.
7. Grow at 37 °C with shaking at 250 rpm.
8. Induce at $OD_{600\ nm}$ ~ 0.9 by the addition of isopropyl b-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM.
9. Harvest cells eight hours post-induction.
10. Analyze protein production by SDS-PAGE.

Diminish lag time by > 60% and the number of hours required to attain high cell density by > 45% with the inclusion of 10% ISOGRO in M9.

For more information, please contact:
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