



ISOTEC® Stable Isotopes

Maximize recombinant protein yields with ISOGRO®

- Substantially increase recombinant protein expression levels using ISOGRO as a stand-alone media versus M9 media.
- Save time and money by using ISOGRO growth media to shorten production time.
- Stable isotope label sufficient amounts of protein to permit the achievement of high quality NMR spectra of difficult to express proteins.
- As a standard quality control measure, the suitability of each batch of ISOGRO as a culture medium is determined by comparison with a LB growth curve.

A 39 µM sample of p38 alpha was produced from 50 mL of culture as seen below:

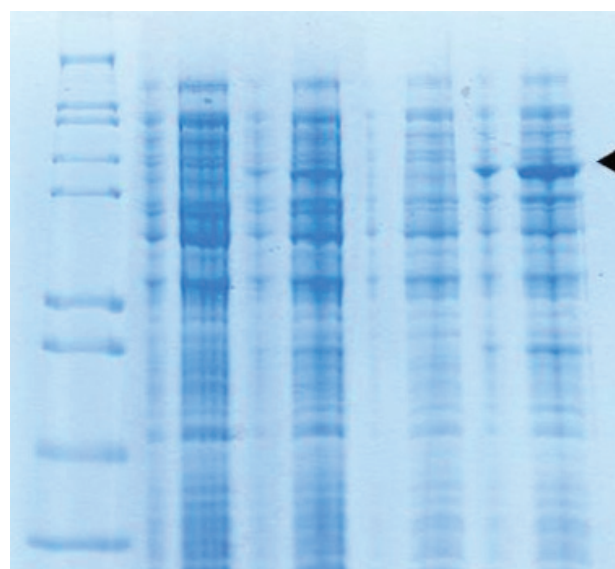


Figure 1. SDSPAGE of p38 growth. Left to right – Molecular weight marker, uninduced minimal media, induced minimal media, uninduced ISOGRO, induced ISOGRO; black arrow – p38 alpha.

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

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

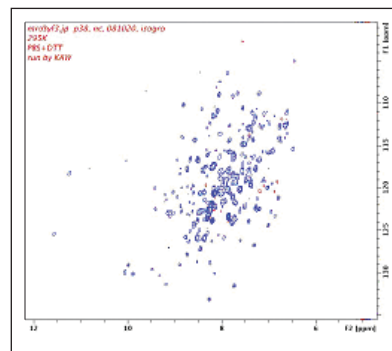


Figure 2. ¹H-¹⁵N TROSY spectrum of [¹³C,¹⁵N] p38 alpha collected at 700 MHz. Data provided by Dr. Jeffrey W. Peng, Dept. of Chem/Biochemistry, Univ. of Notre Dame, Notre Dame, Indiana.



Procedure for Growing *E. coli* using ISOGRO® Powder p38 alpha expression

- Dissolve 0.5 g of ISOGRO powder into 45 mL of filtered, sterilized water.
- Make stock solutions of the following salts and use the listed quantities in the preparation:
- Adjust the pH to 7.0 with NaOH and bring the solution up to 50 mL.
- Add 2.5 mg ampicillin to the solution.
- In the positive flow hood, pass the solution through a 0.22 um filter and transfer the filtrate to a sterilized 500 mL flask. (Used 2 filters and 2 syringes).
- Inoculate the culture with a loop of *E. coli*.
- Shake at 37 °C at 200 rpm.
- OD_{600 nm} = 0.36 at 6.5 hours.
- OD_{600 nm} = 1.2 at 8 hours.
- Save an aliquot for gel: 0.4 mL of 1:10 dilution from OD, spin down and save pellet in -20 °C.
- Change the shaker temperature to 20 °C, let the cells equilibrate for 30 minutes.
- Induce using 25 uL 1M IPTG and incubate overnight at 20 °C at 225 rpm.
- OD_{600 nm} = 2.0 at 14 hours after induction.
- Save an aliquot: 1:10 dilution from OD, spin down, and save pellet in -20 °C.

	Stock	Amount
K₂HPO₄	100g/L	0.9 mL
KH₂PO₄	50g/L	1.4 mL
MgSO₄	50g/L	1.0 mL
CaCl₂	37g/L	15.0 uL

ISOGRO usage results in the production of micromolar amounts of protein from 50 mL of culture.

For more information, please contact:

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*Accelerating Customers'
Success through Innovation and
Leadership in Life Science,
High Technology and Service*

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