

Growth Medium

ISOGROM™ Powder (1)

ISOGRO™ Powder is prepared from algal cells which are grown under appropriate isotope feed conditions. The suitability ISOGRO™ as a culture medium has been demonstrated in our labs by growing *E.Coli* strain W3110, ATCC 27325, in comparison with ATCC LB broth under identical conditions, with no significant differences in the two curves. Typical results are reported in the graph on the next page. **We recommend that customers try a small quantity of ISOGRO™ to determine its suitability for their specific applications.** Users should determine the ideal composition required for their particular organisms and add the growth factors and/or adjust conditions individually. Isotec sells ISOGRO™ in powder form rather than liquid because we believe there is less chance of bacterial contamination during handling, and because it gives our customer maximum control over salt and other nutrient concentrations.

| Compound | Atom % | Typical Batch Analysis of ISOGRO™ Powder | |
|--|---|--|--|
| ISOGRO™ Powder Growth Medium (unlabelled) | | Isotopic Enrichment | 99 atom % ¹³ C and / or 99 atom % ¹⁵ N and / or 97-99 atom % D |
| ISOGRO™ - ¹³ C Powder Growth Medium | 99 | Total Carbon | ~ 28 % |
| ISOGRO™ - ¹⁵ N Powder Growth Medium | 99 | Total Nitrogen | ~ 9 % |
| ISOGRO™ - ¹³ C, ¹⁵ N Powder Growth Medium | 99 ¹³ C 99 ¹⁵ N | Salt | ~ 30 % |
| ISOGRO™ - ¹³ C, ¹⁵ N, D Powder Growth Medium | 99 ¹³ C 99 ¹⁵ N 97-99 D | Water | ~ 3 % |
| | | Glucose | ~ 1 - 3 % |

Typical Procedure for Growing *E. coli* Using ISOGRO™ Powder

To prepare 100 ml ISOGRO™ medium, we suggest the following:

1. Dissolve 1,0 g of ISOGRO™ Powder in about 90 ml of Millipored water.
2. Make stock solutions of the following salts and use the quantities indicated in the medium preparation:

| | Salt | Conc. of Stock Soln. | Qty. / 100 ml Medium |
|--|--------------------------------------|----------------------|----------------------|
| | K ₂ HPO ₄ | 100 g / L | 1.8 ml |
| | K ₂ HPO ₄ | 50 g / L | 2.8 ml |
| | MgSO ₄ | 50 g / L | 2.0 ml |
| | CaCl ₂ · H ₂ O | 37 g / L | 30 µl |

3. Adjust pH to 7,0 with NaOH and bring solution up to 100 mL with Millipored water.
4. Pass the solution through a 0,22 µm filter and transfer the filtrate to an autoclaved shaker flask (for example: 50 mL medium in a 500 mL flask).
5. The culture is inoculated with a loop of *E. coli* which has been maintained on a nutrient agar slant.
6. Shake the culture flask in a 37 °C water bath.
7. The absorbance of the culture is measured at 600 nM with a 1 : 3 dilution into water.

Growth Medium

ISOGRO™ Powder (2)

Comparison Medium

The LB Broth used as a comparison medium is made up as described in the ATCC Catalog. It consist of the following:

| | Item | Amount |
|--|----------------------------|--------|
| | Typtone (Difco 0123) | 1.0 g |
| | Yeast Extract (Difco 0127) | 0.5 g |
| | Sodium Chloride | 1.0 g |
| | Millipored Water | 100 g |

This solution is sterilized by filtration and the flasks treated in the same manner as for the labelled medium. Isotec incorporates this comparison with commercial LB Broth medium into our quality control testing of each batch of ISOGRO™ produced.

The graphs below demonstrate that culture media incorporating Isotec's ISOGRO™ Powder does support *E. coli* cell growth as efficiently as commercial unlabelled growth medium (LB Broth) does.

Researcher's specific applications do vary; so our growth conditions should serve as a guideline.

In general, we anticipate ISOGRO™ would be used as a supplement together with D-Glucose-¹³C₆ (99 atom %) or additional ¹⁵N-ammonium salts, for maximizing results.

