IG® Autoinduction Medium

Manual

Catalog #	1722-1L	1722Pd-1L
Package Type & Size	Liquid, 1.0 L	40g Powder, for 1.0 L

Important!

* Immediately inspect packages
* Store at 4°C upon receipt

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Intact Genomics, Inc.

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Description:

Intact Genomics (IG®) Autoinduction Medium promotes high-yield growth of E. coli and high-level expression of T7 promoter-regulated recombinant proteins without time-consuming steps such as monitoring optical density (OD) or adding induction components like IPTG.

Unlike traditional LB-IPTG induction systems, IG® Autoinduction Medium allows regulated protein expression in any expression system that is solely inducible by IPTG from E. coli strains containing a fully functional lac operon.

We have also developed an Autoinduction DE3 strain. Both chemical (Catalog # 1065) and electroporation (Catalog # 1265) competent cells are available. These cells have been used to test a broad variety of protein targets resulting in robust protein expression and production (**Figure 1**). Protein expression begins automatically at very high cell density after inoculation with a single colony or starter culture. After an incubation time of ~7 hours, the culture is shifted to a lower temperature overnight for continued growth and protein expression under standard conditions.

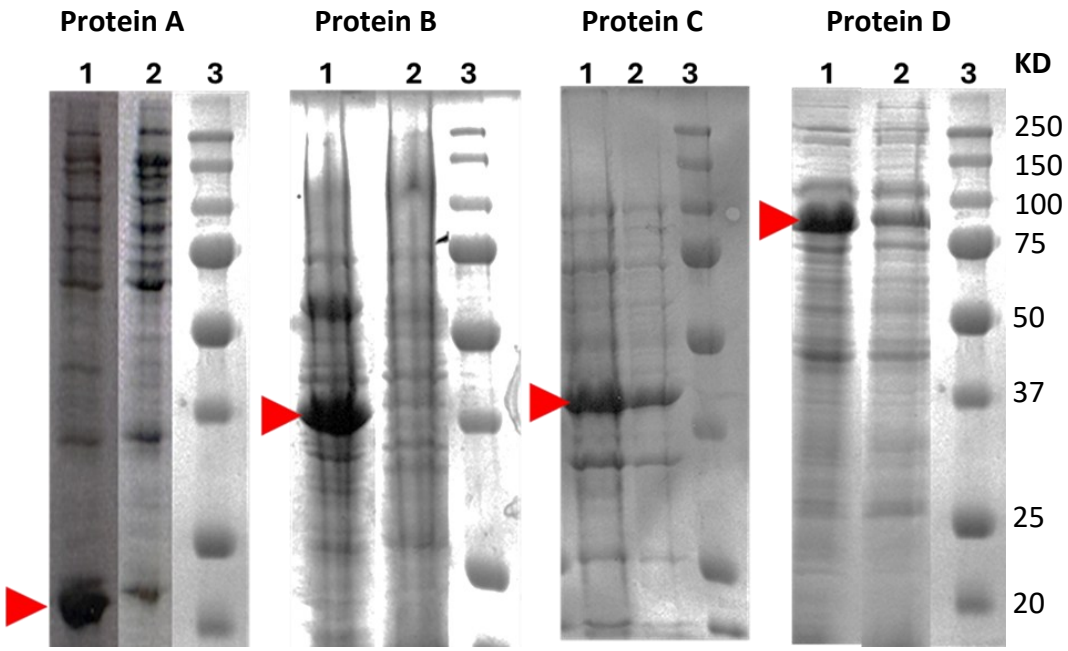

Figure 1. Lane 1: The proteins were expressed in IG® autoinduction medium
Lane 2: The proteins were expressed in LB with IPTG
Lane 3: Protein molecular weight marker

Four examples of the genes of different recombinant proteins that were cloned into a common pET30b vector and were used to transform IG® AutoInduction DE3 competent cells. Each expression culture (100µL of cells) was collected, cells were lysed, and cell extracts were analyzed on a Coomassie Blue stained SDS-PAGE gel with ~ 11% Bis- buffer system. Autoinduction lanes (1) show more robust protein expression (~3-fold to 10-fold) higher in these examples.

Product Components:

Component 1 (in one of two optional formats)

1-L: liquid, 950 mL, ready-to-use;

1-P: powder, 40 grams

Component 2: 50mL

Package Formats: Component 1-L: bottle; Component 1-P: bag; Component 2: bottle

Shipping condition: Room Temperature

Storage: 4°C

Vectors and Bacterial Strains

IG® Autoinduction Medum is designed for inducible T7 promoter-regulated protein expression systems. IG® Autoinduction DE3 strain is optimized and shows a higher success rate using IG autoinduction medium and simple growth conditions than other types of BL21(DE3) and derivate strains below. T7 promoter-regulated expression vectors (such as pET) are suitable for cloning your gene of interest. E. coli expression strains that contain a fully functional lac operon (including lacY and lacZ) such as BL21(DE3), and BL21(DE3)pLysS strains can be used with IG® autoinduction medium.

IMPORTANT TIPs: Some *E.coli* expression strains including T7-regulated expression systems with a partially functional lac operon (such as NEB T7 expression strain), and E. coli cloning strains such as DH10B, DH5alpha, etc. CANNOT be used for autoinduction of protein expression.

IG Autoinduction Medium Preparation

IG® Autoinduction Medium (1L medium) component 1 comes with two formats: liquid Component 1-L (ready-to-use), and dry power, Component 1-P. Component 2 is a sterile liquid and ready to be added into Component 1.

For Liquid, Component 1-L (ready to use):

Inside a clean/sterile hood, use aseptic technique to add Component 2 (50mL) of Autoinduction Medium, selective antibiotic of choice (e.g. 50 µg/mL kanamycin) depending on the resistance gene in your vector to Component 1 (950 ml), mix well. Your IG autoinduction medium is completed and ready to use.

Notes: If a precipitate is visible in Autoinduction Medium Component 2, it can be re-dissolved by warming at 37°C and gently shaking or swirling the bottle until the precipitate is gone. Then add it to Component 1 as described above. The formation of precipitate will not affect product performance. Keep unused complete medium at 4°C for up to 3-4 weeks.

For Dry Powder, Component 1-P:

1. Completely dissolve the entire bag of Autoinduction Medium Component 1-P in 950 mL of de-ionized water in an autoclavable flask.
2. Autoclave on a liquid cycle for 15 minutes. Cool media to room temperature.
3. Inside a clean/sterile hood, use aseptic technique to add Component 2 (50mL) of IG Autoinduction Medium, selective antibiotic of choice (e.g. 50 µg/mL kanamycin) depending on the resistance gene in your vector to Component 1 (950 mL) , mix well. Your IG autoinduction medium is completed and ready to use.

Protocol:

Tips: Culture tube/flask/vessel size and culture conditions

- Optimal aeration of the culture during incubation is critical to achieve high-density growth.
- IG Autoinduction Medium expression cultures must be incubated with vigorous shaking at 250 rpm in an appropriately sized tube/flask/vessel.
- For small-scale expression cultures, add 1 mL complete IG Autoinduction Medium to a sterile 15 mL culture tube.
- For a larger volumes in non-baffled/ baffled flask, use 10%-20% flask volume of complete IG Autoinduction Medium.
- Culture at 37°C is generally recommended for maximum growth of Autoinduction Medium expression cultures. Depending on your protein, culture at 30°C or Duel-temperature with start culture (see Enhanced protocol on page 6) may further improve protein folding, solubility and or yield.

Starter culture: A starter culture is recommended for Autoinduction Medium expression culture volumes >100 mL. To make a starter culture, inoculate 1/15 of the final culture volume of LB media with selective antibiotic with a fresh colony or glycerol stock and grow overnight with shaking at 30°C. For expression culture volumes of <100 mL, directly inoculate Autoinduction Medium with a fresh colony or glycerol stock.

Simple protocol:

1. Add complete IG Autoinduction Medium to sterile tubes or flasks according to the culture vessel size recommendations above.
2. Use sterile technique to inoculate the colony directly into the medium. Be sure to patch the colony onto a separate selective plate if needed. If you are using a starter culture, add the entire volume to the Autoinduction Medium culture flask using aseptic technique.
3. Cap tube or flask and secure in an incubator.
4. Incubate at 37°C or 30°C with vigorous shaking (250 rpm) for 18–24 hours.

Enhanced protocol:

Protein expression cells can be grown at 37°C or 30°C, for ~7 hours ($O.D._{600}$ = ~7.0.) at 250rpm from a starter culture then shift to 18°C autoinduction overnight for 13-19 hours. This protocol is for specific applications related to protein solubility and toxicity or robust yield of both cells and target protein.

Day 1: 1. Inoculate 10-200 mL LB/antibiotic with an expression colony and incubate overnight at 30°C with vigorous shaking (250 rpm).

2. Place the appropriate amount of complete Autoinduction Medium/antibiotic at 37°C or 30°C to pre-warm it ~1 hour (ideally, not required).

Day 2: 1. Inoculate pre-warmed, complete Autoinduction Medium /antibiotic with an overnight seed culture at a 1:15 dilution.

2. Incubate the culture with vigorous shaking (250 rpm) at 30/37°C for ~7 hours or until $O.D._{600}$ = ~7.0. When measure $O.D._{600}$ for a culture >1.0 OD, dilute an aliquot of the expression culture 1:10 in water or fresh media (blank the spectrophotometer with the same diluent), measure the $O.D._{600}$, may help to obtain an accurate OD value.

3. Transfer the culture to an incubator set at 18°C with vigorous shaking (250 rpm), incubate for 13-19 hours.

Days 3: Harvest culture and analyze recombinant protein expression.

Important Tips: An overnight starting culture in rich medium such as LB or 2xYT at 37°C usually reached saturation by the next morning. Which might result in the plasmid instability because of the basal leakage of the T7 expression system and poor yield of target protein. To avoid this issue, starting culture in a rich medium for ~5 hours at 37°C or overnight at 20–25°C or 30°C until the $O.D._{600}$ is between 3-5 in LB or 5-7 in 2xYT is strongly recommended.

Optimize Lysis Conditions:

Under the growth conditions specified above, E. coli expression or IG® Autoinduction DE3 strains usually grow to 3-5 times or more optical density in Autoinduction Medium compared to traditional medium.

To ensure efficient bacterial lysis using your method of choice, you will need to estimate the amount of E. coli cells in your expression culture. Dilute an aliquot of the expression culture 1:10 in water or fresh media (blank the spectrophotometer with the same diluent), measure the $O.D._{600}$ to calculate the approximate cells/mL, and optimize your lysis conditions accordingly.

Related Products:

- IG® Autoinduction Chemically Competent Cells (Cat.#: 1065)
- IG® Autoinduction ElectroCompetent Cells (Cat.#: 1265)
- ig® 5-Alpha Chemically Comp. Cells (Cat.# 1031-12)
- BL21(DE3) Electroporation Comp. Cells (Cat.# 1212-12)
- T4 DNA Ligase (Cat.# 3212)
- i7® High Fidelity DNA Polymerase (Cat.# 3254)
- igFusion™ Cloning Kit (Cat.# 4111)

Ordering Information:

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.
  Email: **sales@intactgenomics.com**
  Phone: (314) 942-3655 **|** Toll-free : 855-835-7172 **|** Fax: (314) 942-3656
- Order via our distributors.

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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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