Frequently Asked Questions

Part # TR-1050
04/05

1. What if my protein does not express?

- Try using a higher concentration of The INDUCER™.
- Express the protein using IPTG and normal conditions to determine if the bacterium still contains the plasmid.
- The expression plasmid used must contain a T7*lac* promoter.

2. What if my protein is still insoluble?

- Try using less of The INDUCER™, 1.0-2.0 grams/liter.
- Reduce the incubation temperature.
- Add at least 1mM DTT to the lysis buffer or use another reducing agent such as b-mercaptoethanol.

3. My protein expresses but the yield is low compared with IPTG

- This is expected. The INDUCER™ will not yield as much total protein or protein of interest but a greater percentage of the total should be in the soluble fraction.

MoleculA	
22863 Bryant Court, Suite 102	
Sterling,VA 20166 USA	
Telephone	703.481.9207
Toll Free	800.381.0856
Fax	703.481.9209
Internet	www.molecula.com

moleculA
A Calbiotech Company

The Inducer™

I. Description

The INDUCER™ is a novel IPTG replacement that increases the level of soluble, heterologously expressed protein in *E. coli* containing the T7*lac* promoter. By decreasing the rate of expression, The INDUCER™ enables expressed protein to fold properly and remain soluble thereby reducing the formation of insoluble inclusion bodies.

Perhaps the easiest method for generating significant amounts of soluble protein in *E. coli* is to create conditions that reduce the rate of expression. A slower rate of expression can be achieved by reducing incubation temperatures and IPTG concentrations, but these manipulations, as most researchers realize, yield only marginal increases in the amount of soluble protein. A better alternative for reducing expression rates is to use The INDUCER™ since it has a lower binding affinity for the *lac*I repressor compared with IPTG. As a consequence, the rates of expression are slower with The INDUCER™ resulting in an increased level of soluble protein.

II. Working Concentration

The INDUCER™ is provided at a concentration of 0.2 g/ml. Determining the concentration that favors the formation of soluble protein must be determined experimentally, but usually ranges from 2 to 6 g/L.

III. Determining INDUCER™ concentration for optimal solubility

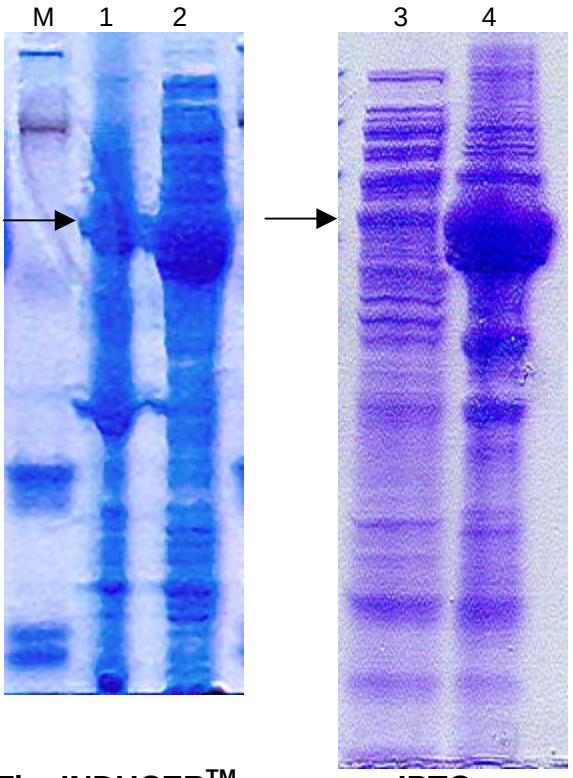
Pick individual bacteria colonies into 4-6 ml LB or NYZ medium plus the appropriate antibiotic. Incubate in a shaker (250 RPM) at 37° C overnight or until the culture obtains an O.D.= 0.6-0.8 at 600 nm. In the morning, add 2-3 ml of the starter culture to 600 ml of medium containing the same antibiotic. Incubate the culture at 37° C until an O.D.= 0.6-0.8 is obtained. Cool the culture on ice to between 15-20° C then split the volume equally into three flasks. Add The INDUCER™ to a final concentration ranging from 1.0 - 6.0 g/L. Also add the same amount of antibiotic to the culture that was used initially. Incubate in a shaker (RPM 125) at room temperature for at least 12 hours or overnight. After incubation, lyse cells and isolate the soluble and insoluble fractions according to standard protocols. Determine the amount of soluble verses insoluble protein by visualizing whole cell lysate fractions using denaturing gel electrophoresis and the appropriate staining methods.

IV. Additional Information

The INDUCER™:

Part No.	Size (ml)	Cost (USD)
TR-1050-100	100	$75.00
TR-1050-500	500	$285.00

Storage Temperature: 2-8°C.



The INDUCER™, 2g/L **IPTG, 1 mM**

Figure 1 The 52 kDa protein of interest was expressed in *E. coli* using a pET T7*lac* expression vector (Novagen). Cultures were grown at 37°C to approximately 0.7 O.D. units. Cultures were cooled to 17 °C and induced with either 2g/L of The INDUCER™ or 1mM IPTG (ICN, Costa Mesa, CA) for 14 hrs at room temperature. Soluble and insoluble whole cell lysate fractions were isolated and loaded on to a NuPAGE Novex 12% Bis-Tris gel (Invitrogen). Following electrophoresis, the gel was stained with the Colloid Blue Staining Kit (Invitrogen).
M: Multi mark (Invitrogen)
Lane 1: The INDUCER™ soluble whole cell lysate
Lane 2: The INDUCER™, insoluble whole cell lysate
Lane 3: IPTG, soluble whole cell lysate
Lane 4: IPTG, insoluble whole cell lysate