GROWCELLS.COM
A GROWING CULTURE...

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I. Description

The HyperINDUCE ™ 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, HyperINDUCE ™ 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 HyperINDUCE™ 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 HyperINDUCE ™ resulting in an increased level of soluble protein.

II. Working Concentration

The HyperINDUCE™ is provided at a concentration of 2g/L. 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 HyperINDUCE™ concentration for optimal solubility

Pick individual bacteria colonies into 4-6 ml, HyperGRO, 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 HyperINDUCE ™ 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

Try our Induction ready HyperGRO broth that already has HyperINDUCE in the right concentration.

Troubleshooting: Frequently Asked Questions

1. **What if my protein does not express?**
   - Try using a higher concentration of The Inducer™.
   - Run verses normal conditions to see if plasmid is still in the bacteria.
   - 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 more DTT to the lysis buffer or another reducing agent.
3. **My protein expresses but the yield is low?**
   - This is expected, The Inducer™ will not yield as much total protein or protein of interest but more should appear in the soluble fraction.