

## InduX™ Medium Powder

Cat No: 40145

Contents: powder for one liter medium, Adds I, and Adds II

Storage: room temperature for six months

### Description

InduX™ is a medium for toxic protein expression in E.coli cells. The medium is intended to use with DetoX™ medium. Cells should be grown in DetoX™ first to reach induction OD, then switch the medium to InduX™ for induction.

The E.coli cells can grow to a density of 20 to 30 (OD600) in this medium which is significantly higher than the density that can be obtained by a regular medium such as LB. Five to 20 times recombinant protein may be produced compared with a regular medium.

The protein can be induced at early log phase which is OD600=5 to 10 for this medium. It can also be induced at saturation OD600=20 to 30.

Higher concentration of antibiotic and inducer is needed for higher cell density. We normally use 200 ug/ml ampicillin for selection. Our InduX™ medium contains inducer. No extra inducer is needed if the protein is induced by IPTG.

### Aeration

Good aeration is critical for high cell density and protein expression. Please check the aeration of the incubation room, incubator, and the container.

After cells reach OD600=10, they will need sufficient amount of oxygen to reach higher density. Low shaking speed cannot support cell growth over OD600=20. High shaking speed may result in medium spilled out. Please note maximum shaking speed is different for each type of container with defined volume of medium.

At recommended shaking speed, all clamps and containers should be secured on the platform. Balanced loading will increase incubator life especially when large volumes are used.

Incubation room needs to be sufficiently ventilated.

Ventilation fans of many incubators may require temperature setting. Therefore room temperature incubation (25 °C) will still need to set temperature at 25 °C to keep the fan on.

Container cover cannot be closed. Use the cover allowing best ventilation possible. After OD600=10, the container cover may be removed to maximize aeration. We never encounter any cross-contamination at this or higher cell density.

### Protocol

1. Dissolve one bag of powder in 900 ml DI H<sub>2</sub>O with heating and stirring. Add 20 ml 50% glycerol (not provided) and DI H<sub>2</sub>O to 1000 ml. Sterilize the medium. Add 0.1% Adds I, Adds II, and appropriate antibiotic just before use. Shake the medium well before aliquoting\*.

2. Add InduX™ medium to the cell pellet which were grown to induction OD in DetoX™ medium.

3. Induce the cells at different temperature for different time period. Cells can be induced at temperatures between 15 to 37 °C. The lower the temperature is, the longer induction time is needed. 24 to 36 hours may be needed for cells induced at 16 °C.

\* Antifoaming agents do not dissolve in the medium. Shake well if foams are not desired. This is important for baffled flasks. Under most conditions, there is no foam in tube cultures and there are less foams in regular flasks.

E.coli cell density in this medium will depend on the toxicity of the recombinant protein. If the protein is not toxic to the host, the cells can grow to a density of 30 to 40. Some toxic protein can decrease cell density. In this case, it is best to induce the cells at high density (OD600=20 to 30) to get reasonable yield.

### Toxicity

If some cells can reach high density (>OD600=20) while others cannot under the same condition. The proteins encoded by the plasmids in the low density cells may be toxic to the host. Our detoxification cell strains may be needed to express these proteins. Combining our detoxification cell strain with our medium will increase cell density and protein expression significantly.

### Regular verses Baffled Flasks

Baffled flasks generate better aeration at larger volume with low shaking speed. Larger medium volume (2 to 4 times) can be used at low shaking speed in baffled flasks. However some baffled flasks produce excessive foams which act as barriers for cells to access oxygen. Cell density in this kind of baffled flasks can rarely reach density over OD600=30.

### Induction Temperature

After cell density reaches 10, the cells can be grown at temperatures between 16 to 37 °C. The lower the temperature is, the longer growth time will be needed. 24 to 48 hours may be needed for cells grown at 16 °C. Overnight growth (>14 hours) may be performed at 25 to 37 °C. Lower temperature may increase solubility and decrease toxicity for some proteins.