

InduX™ Medium

Cat No: 40141, 40142, 40143, 40144

contents: sterilized 1x liquid medium, Adds I and Adds II

Storage: room temperature

Description

InduX™ Medium is a designed for high yield expression of toxic protein in E.coli cells. The medium is intended to use with DetoX™ or ProGro™ medium. Lactose is included in medium.

The E.coli cells can grow to a density up to 40 (OD₆₀₀) which is significantly higher than the density that can be obtained by a regular medium such as LB. Ten to 20 times recombinant protein may be produced compared with conventional medium.

The protein can be induced at early log phase which is OD₆₀₀=2 to 5 for these media. It can also be induced near saturation OD₆₀₀=10 to 20. Higher yield will be obtained at higher cell density.

Higher concentration of antibiotic is needed for higher cell density. We recommend 200 ug/ml ampicillin for selection.

Aeration

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 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 should be removed if highest cell density is desired. We never encounter any cross-contamination at this or higher cell density.

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 25) to get reasonable yield.

Antifoaming

Antifoaming agents do not dissolve in the medium and they will not affect cell grow or protein expression. Shaking well is critical before using or aliquoting if foams are not desired during and after culture.

Protocol

1. Add appropriate amount of antibiotic, Adds I and Adds II (0.1% v/v) to the InduX medium immediately before use. Shake the medium well before using or aliquoting. Add InduX™ medium to the cell pellet which were grown to induction OD in DetoX™ medium.

2. No IPTG or lactose is needed in InduX™ medium if the protein is induced by IPTG*. Grow the cells at different temperature for different time period.

3. Cells can be induced at temperatures between 10 to 37 °C at 350 rpm. The lower the temperature is, the longer induction time is needed. 24 to 36 hours may be needed for cells induced at 16 °C. We recommend to induce toxic proteins at a temperature lower than 37 °C.

temperature	15 °C	25 °C	37 °C
recommended induction time	10-36 hours	5-16 hours	1-5 hours

Protein induced in the InduX™ medium is often more soluble compare to that in other media.

*If the protein is induced other than IPTG, the specific inducer is needed for the induction.

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 medium may be spilled out in some baffled flasks at 350 RPM or higher speed. Lower volume or lower shaking speed should be used for these flasks.

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. Low temperature may increase protein solubility and decrease protein toxicity.

Aseptic operation:

It is best to use all of the medium once it is opened. Wash both hands and clean the working surface by 70% ethanol. Do not cough, sneeze, or breath into the medium. Hold breath when opening the medium and quickly close it after transferring the medium out. Avoid opening the medium in places such as bacterial culture rooms. Use a biological hood if contamination persists.