

DetoX™ Medium Powder

Cat No: 40136

Contents: powder, Adds I, and Adds II for 10 liter medium

Storage: room temperature for six months

Description

DetoX™ is a medium for toxic protein expression in E.coli cells. The medium is intended to use with InduX™ 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 (OD_{600}) in this medium. 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 $OD_{600}=5$ to 10 for this medium. It can also be induced near saturation $OD_{600}=20$ 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 $OD_{600}=10$, they will need sufficient amount of oxygen to reach higher density. Low shaking speed cannot support cell growth over $OD_{600}=20$. 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 $OD_{600}=10$, the container cover should be removed if highest cell density is desired.

We never encounter any cross-contamination at this or higher cell density.

Antifoaming

Antifoaming agents do not dissolve in the medium and they will not affect cell grow or protein expression. Shaking well

Protocol

1. Dissolve one bag of powder in 1.8 liter dI H₂O with heating and stirring. Add 20 ml 50% glycerol (not provided) and dI H₂O to 2 liter. Sterilize the medium. Add 0.1% Adds I, 0.1% Adds II and appropriate antibiotic before use*.

2. Inoculate at 1:100 for most E.coli strains. The medium volume should be 1/8 of a flask or 1/10 of a tube volume or less. For example, 250 ml or less should be used in a 2-liter flask. Make sure the container is sufficiently ventilated.

3. Grow the cells to appropriate density. The cells need to be diluted to $OD_{600} \leq 0.3$ to get accurate reading (about 100x dilution). Spin down the cells. Add InduX™ medium and appropriate antibiotic.

4. Induce the cells at different temperature for different time period. Cells can be induced at temperatures between 10 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 15 °C or lower temperature.

*The contents are not mixed homogenously and they cannot be prepared in smaller volumes (<2 liters).

is critical before using or aliquoting if foams are not desired during and after culture.

Toxicity

If some cells can reach high density ($>OD_{600}=20$) 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 $OD_{600}=30$.

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 protein solubility and decrease protein toxicity.