

DetoX™ Medium

Cat No: 40131, 40132, 40133, 40134
contents: liquid medium, 1000X Adds I and Adds II
Storage: room temperature

Description

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

The E.coli cells can grow to a density of 30 to 50 (OD_{600}) 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 conventional medium.

The protein can be induced at early log phase which is $OD_{600}=2$ to 5 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, 0.5 to 2 mM IPTG and 2 to 10 mM lactose for induction.

Aeration

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

Protocol

1. Add appropriate amount of antibiotic, 0.1% v/v Adds I and II immediately before use. Shake the medium well before using or aliquoting. Inoculate at 1:100 for most E.coli strains. The medium volume should be 1/4 of a flask or 1/10 of a tube volume or less. For example, 500 ml or less should be used in a 2-liter flask. Make sure the container is sufficiently ventilated.

2. Grow the cells to appropriate density. The cells need to be diluted to $OD_{600} \leq 0.3$ to get accurate reading.

3. Spin down the cells. Add appropriate volume of InduX™ medium with appropriate antibiotic. Induce the cells at different temperature for different time period.

Flask	Regular		Baffled		Tube
RPM	400 to 450		350 to 400		350 to 400
Volume	1/8	1/4	1/4	1/2	1/10
OD_{600}	35	25	35	25	35

Cells can be induced at temperatures between 15 to 37 °C. Above induce time is good for 30 to 37 °C. The lower the temperature is, the longer induce time is needed. 24 to 36 hours are needed for cells induced at 16 °C. Overnight induction (>14 hours) is well tolerated in this medium with lactose as an inducer.

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 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. Lower temperature may increase protein solubility and decrease some protein toxicity.