

AutoX™ Medium Powder

Cat No: 40126

Contents: powder, Adds I, and Adds II (for 5 x 2 liters)

Storage: room temperature for six months

Description

AutoX™ is a medium for high yield and automatic expression of recombinant protein in E.coli cells.

The medium contains trace metals, minerals, and vitamins which may serve as ligands, co-factors or prosthetic groups for proteins.

The E.coli cells can grow to a density of 40 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 a regular medium.

The recombinant protein will be automatic expressed after cells reach $OD_{600}=10$. No IPTG is needed for the induction. If the inducer is not IPTG, specific inducer must be added to induce the protein expression. We normally use 200 ug/ml ampicillin for selection. Some expression may need additional 200 ug/ml ampicillin after the culture reaches $OD_{600}=10$.

50% glycerol should be used for accurate measurement.

Aeration

Good aeration is critical for high cell density and auto induction. 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 $OD_{600}=20$. Higher shaking speed than those specified in the table will 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 for large volume. 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 1% glycerol (not provided) and dI H₂O to 2 liter. Sterilize the medium and cool it to room temperature. 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 at **350-450 rpm** shaking at 37 °C. The higher shaking speed, the higher cell density can be obtained. The cells need to be diluted to $OD_{600} \leq 0.3$ to get accurate reading (about 100x dilution).

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}	45 35	45 35	45

* Adding Adds I and II before cooling to room temperature will cause precipitation and the resulting medium can only reach OD_{600} 20. The contents are not homogenously mixed 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 medium and 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 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 $OD_{600}=35$.

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) should be performed at 25 to 37 °C. Lower temperature may increase protein solubility.