

AutoX™ Medium

Cat No: 40121, 40122, 40123, 40124

Contents: liquid medium, Adds I and Adds II

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 35 to 45 (OD₆₀₀) 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 OD₆₀₀=10. No IPTG is needed for the induction. If the inducer is not IPTG, the recombinant protein will not be induced. Specific inducer must be added to induce the protein expression. We normally use 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. Higher shaking speed than those specified in the table may result in medium spilled out. Please note maximum shaking speed is different for each type of container and incubator 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.

Antifoaming

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.

Protocol

1. Add 0.1% v/v Adds I, Adds II and appropriate amount of antibiotic just before use. 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.

2. 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 OD₆₀₀ ≤ 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

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.

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 medium and cell strains may be needed to express these proteins. Combining our detoxification cell strain with our medium will increase cell density 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 OD₆₀₀=30.

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.