

DNAGro™ Medium Powder

Cat No: 40105

Contents: powder and Adds I for one liter medium

Storage: room temperature

Description

DNAGro™ is a medium for high plasmid yield in E.coli cells. The E.coli cells can grow to a density of 35 to 45 (OD₆₀₀) in this medium which is significantly higher than the density that can be obtained by a regular medium such as LB. Ten to 20 times plasmid DNA may be produced compared with a regular medium. High cell density requires high concentration of antibiotic. We normally use 200 ug/ml ampicillin for selection. For some high copy number plasmids, additional 200 ug/ml ampicillin may be needed after the culture reaches OD₆₀₀=10.

Plasmid DNA quantity may exceed column capacity. In this case, larger capacity column should be used.

Glycerol should be prepared at 50% to ensure its accurate addition.

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 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.

Antifoaming

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

Protocol

1. Dissolve one bag of powder in 900 ml dI H₂O with heating and stirring. Add glycerol (not provided) to 1% and dI H₂O to 1000 ml. The medium will be sterilized if it is boiled for 3 minutes or microwaved to bubbling for 30 seconds. Add 0.1% (v/v) Adds I and appropriate amount of 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. 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 overnight. 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

4. Harvest the cells and perform plasmid prep.

*Adding Adds I when preparing the medium may produce precipitation and high cell density cannot be achieved.

Toxicity

If some cells can reach high density (>OD₆₀₀=20) while others cannot under the same condition. The plasmid or protein encoded by the plasmid in the low density cells may be toxic to the host. Our detoxification medium and cell strains may be needed to produce this plasmid. 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.