

## ProGro™ Medium Powder

Cat No: 40116

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

Storage: room temperature

### Description

ProGro™ Medium is a designed for high yield protein expression 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 20 to 30 (OD<sub>600</sub>) 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 protein can be induced at early log phase which is OD<sub>600</sub>=5 to 10 for this medium. It can also be induced at OD<sub>600</sub>=15 to 20.

Higher concentration of antibiotic is needed for higher cell density. We normally use 200 ug/ml ampicillin for selection and same concentration of ampicillin is added at induction.

### 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<sub>600</sub>=10, they will need sufficient amount of oxygen to reach higher density. Low shaking speed cannot support cell growth over OD<sub>600</sub>=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 when large containers 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<sub>600</sub>=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.

### Protocol

1. Dissolve one bag of powder in 1.8 liter\* dI H<sub>2</sub>O with heating and stirring. Add 40 ml 50% glycerol (not provided) and dI H<sub>2</sub>O to 2 liters. Sterilize the medium. Add Adds I, Adds II and appropriate antibiotic just 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.

3. Grow the cells to appropriate density in a 2 to 3 liter flask 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<sub>600</sub> ≤ 0.3 to get accurate reading (at 100x dilution).

4. Add appropriate amount of IPTG or inducer at OD<sub>600</sub>=5 to 10. Induce the cells at different temperature for different time period.

\*The contents cannot be mixed homogenously and they cannot be prepared in smaller volumes. Antifoaming agents do not dissolve in the medium. Shaking well is critical before aliquoting if foams are not desired during and after culture.

### Toxicity

If some cells can reach high density (>OD<sub>600</sub>=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<sub>600</sub>=35.

### Induction Temperature

After cell density reaches 10, the cells can be grown at temperatures between 10 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 15 °C. Lower temperature may increase protein solubility and decrease some protein toxicity.