

SecPro™ Medium Powder

Cat No: 40165

Contents: powder for one liter medium, Adds I, and Adds II

Storage: room temperature for six months

Description

SecPro™ is a medium for high yield secretory protein expression in E.coli cells.

The E.coli cells can grow to a density of 20 to 30 (OD₆₀₀) which is significantly higher than the density that can be obtained by a regular medium such as LB. Over 100 times recombinant protein may be secreted compared with a regular medium.

The protein can be induced at early log phase which is OD₆₀₀=2 to 5 for this medium.

Because of high cell density, higher antibiotic concentration may be needed. We normally use 200 ug/ml ampicillin for selection.

Low inducer concentration will promote protein secretion.

We normally use 30 to 80 uM IPTG for induction.

Adding Triton X100 (not provided) will further increase secretion 20 to 40 times.

Aeration

Good aeration is critical for high cell density. Please check the aeration of the incubation room, incubator, and the container.

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 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 (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.

Secretion

If the protein secretion is still low with our SecPro™

Protocol

1. Dissolve one bag of powder in 900 ml DI H₂O with heating and stirring. Add 20 ml 50% Triton X100 and 20 ml 50% glycerol (not provided). Add DI H₂O to 1000 ml. Sterilize the medium. Add 1ml Adds I, Adds II and 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. Make sure the container is sufficiently ventilated.

3. Grow the cells at **300-400 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	350 to 400	300 to 350	350 to 400
Volume	1/8 1/4	1/4 1/2	1/10

Some cell strains may not grow well in this medium because of chemicals dissolving the cell outer membrane and cell wall. In this case, grow the cells in ProGro™ or DetoX™ media and induce the cells in SecPro™ at lower shaking speed in a non-baffled flask will increase the cell viability.

medium. Our secretory cell strain PlusS™ may be needed to express these proteins. Combining our secretory cell strain with this medium may increase protein secretion several hundred times.

Regular verses Baffled Flasks

Because of shearing action of the baffled flasks, regular flasks are recommended for SecPro™ medium.

Growth and Induction media

Highest OD can be achieved if the cells are grown in ProGro™ or DetoX™ media and induced in SecPro™ medium.

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) can be performed at 25 to 37 °C. Lower temperature may increase secretion for some proteins.

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.