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SecPro™ Medium

Cat No: 40161, 40162, 40163, 40164
Contents: sterilized 1X liquid medium, 1000X Adds I and II
Storage: room temperature

Description
ProSec™ is a medium for high yield secretory protein expression in E.coli cells.
The E.coli cells can grow to a density of 15 to 20 (OD_{600}) 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 OD_{600} =2 to 5 for this medium. It can also be induced at OD_{600}= 10 to 20.
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.

Protocol
1. Add 1 ml Adds I, 1 ml Adds II* and appropriate antibiotic just before use. Inoculate at 1:100 for most E.coli strains. The medium volume should be 1/4 of a flask or 1/10 of a tube volume or less. For example, 500 ml or less should be used in a 2-liter flask. Make sure the container is sufficiently ventilated.

2. Grow the cells at **300-400 rpm** shaking at 37 ^{0}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).

3. Induce the cells at appropriate temperature with reduced amount of inducer. We normally use 60 uM IPTG.

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
OD_{600}	30	20	30	20	30

Some cell strains may not grow well in SecPro™ medium. In this case, grow the cells in ProGro or DetoX media and induce the cells in SecPro™ will help the cell viabilility.

Aeration
Good aeration is critical for high cell density. 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 volumes.
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 ^{0}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.

Secretion
If the protein secretion is still low with our ProSec™ 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
Baffled flasks generate better aeration at larger volume with low shaking speed. Larger medium volume (2 to 4 times) 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}=20.

Induction Temperature
After cell density reaches 10, the cells can be grown at temperatures between 16 to 37 ^{0}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 ^{0}C. Overnight growth (>14 hours) should be performed at 25 to 37 ^{0}C. Lower temperature may increase secretion for some proteins.

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.