Succinate Medium A

**Solution A**
prepare just prior to use

shake flask: 0.20 g K$_2$HPO$_4$ and 0.50 g (NH$_4$)$_2$SO$_4$ in 17.4 mL
fermenter: 2.40 g K$_2$HPO$_4$ and 6.00 g (NH$_4$)$_2$SO$_4$ in 64.6 mL

Adjust to pH 7.0 with 20% NaOH prior to autoclaving.

**Solution B**
may be stored in stock
50.0 g/L MgSO$_4$·7H$_2$O

shake flask: 0.40 mL
fermenter: 4.8 mL

**Solution C**
may be stored in stock
15.0 g/L CaCl$_2$·2H$_2$O

shake flask: 1.00 mL
fermenter: 12.0 mL

**Solution D1**
prepare just prior to use

shake flask: 4.00 g glucose in 20 mL
fermenter: 48.00 g glucose in 900 mL

**Solution D2**
prepare just prior to use

shake flask: 1.00 g Yeast Extract and 2.00 g Tryptone in 50 mL
for fermenter: 12.00 g Yeast Extract and 24.00 g Tryptone 100 mL

**Antibiotic solutions**

Ampicillin: freshly dissolve 0.20 g ampicillin in 2.0 mL DI water.
use 100 µL for shake flask and 1.20 mL for fermenter

Kanamycin: use a 40 g/L solution (stored in freezer)
use 100 µL for shake flask and 1.20 mL for fermenter

Chloramphenicol: use a 2.0 g/L solution (stored in freezer)
use 1000 µL for shake flask and 12.0 mL for fermenter

April 18, 2005
**Vitamin Solutions**

biotin: use 0.5 g/L biotin (stored in refrigerator)
use 200 µL for shake flask and 2.4 mL for fermenter

thiamine·HCl: use 1.0 g/L biotin (stored in refrigerator)
use 100 µL for shake flask and 1.2 mL for fermenter

**IPTG Solution**
may be stored in the freezer
1.0 M IPTG

shake flask: none
fermenter: 1.20 mL (1.0 mM IPTG)
           0.60 mL (0.5 mM IPTG)

Autoclave the four solutions (A, B, C, D1, D2) separately. While the shake-flask or fermenter and solutions are still warm, mix Solutions D1 and D2, then Solution C, then Solution B, then Solution A. The antibiotic solution and the vitamin solutions must be added separately by sterile filtration. Solutions are to be added at the following rate:

The shake tube should contain the following solutions:

1) 0.125 g LB in 5.0 mL
2) 0.10 g glucose in 5.0 mL
3) 10 µL freshly prepared ampicillin solution (as described above)

Patch →
10 mL complex media in a shake tube (use all 10 mL to inoculate) →
~90 mL media in a 500 mL shake flask (use all ~100 mL to inoculate) →
1.10L Fermenter (final volume of 1.20 L)
**Composition**

**shake tube**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>tryptone</td>
<td>5.0 g/L</td>
</tr>
<tr>
<td>yeast extract</td>
<td>2.5 g/L</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0 g/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.0 g/L</td>
</tr>
</tbody>
</table>

**shake flask/fermenter**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>40.0 g/L</td>
</tr>
<tr>
<td>Tryptone</td>
<td>20.0 g/L</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>10.0 g/L</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>5.0 g/L</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>2.0 g/L</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.20 g/L</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>0.15 g/L</td>
</tr>
<tr>
<td>Biotin</td>
<td>1 mg/L</td>
</tr>
<tr>
<td>Thiamine·HCl</td>
<td>1 mg/L</td>
</tr>
</tbody>
</table>