Simple defined autoinduction medium for recombinant protein production in *E. coli* T7 expression system

Zhaopeng Li, Wolfgang Kessler, Joop van den Heuvel, Ursula Rinas

**INTRODUCTION**

Protein production using autoinduction is based on diauxic growth of *E. coli* under the dynamic control of lac operon regulatory elements in a medium with mixtures of glucose and lactose. It omits the step of adding inducer to start protein production. Here an easy to use and cost-effective defined autoinduction medium containing glucose, glycerol, and lactose as carbon substrates and NH₄Cl as sole nitrogen source without addition of amino acids and vitamins is presented. The medium was proven to work well for recombinant proteins with different properties in different cultivation scales from microplate to shaker flask and bioreactor scale.

**Autoinduction medium optimization**

**Universal applicability of T-DAB medium**

**Conclusion:**

In conventional test tube and shaker flask, the autoinduction medium composition was successfully optimized.

**OUTLOOK**

- Autoinduction omits the procedures of monitoring the biomass increase during growth and adding inducer to start production and makes the whole procedure easy, economic and reproducible.
- The cultivation reproducibility and stability of chemical defined medium is much better than complex medium containing nutrients which are not defined. The medium developed in this study can be used for heavy isotope labeling or selenium-methionine labeling for protein structure determination through NMR spectroscopy or X-ray crystallography.

**References**


Li, Z., et al., *Simple defined autoinduction medium for high level recombinant protein production for 77-based E.coli expression system*. submitted

---

**Legend to figure:** The target proteins are described in the first row of each SDS-PAGE. Growth medium and induction method are described in the second row. LB: *E. coli* grown in LB medium induced with 0.25 mM IPTG at 23°C. T-DAB: E. coli grown in T-DAB medium at 23°C. In the first row below each SDS-PAGE, W: Whole cellular protein; S: Soluble part of whole cellular protein; I: Insoluble part of whole cellular protein.

**Table:**

<table>
<thead>
<tr>
<th>Cultivation temperature (°C)</th>
<th>OD600</th>
<th>T-DAB medium</th>
<th>LB</th>
<th>T-DAB</th>
<th>LB</th>
<th>T-DAB</th>
<th>LB</th>
<th>T-DAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>0.25</td>
<td>20.0</td>
<td>6.0</td>
<td>20.5</td>
<td>5.4</td>
<td>19.5</td>
<td>7.6</td>
<td>27.4</td>
</tr>
</tbody>
</table>

**Figure:**

- Autoinduction medium optimization was carried out in test tubes and shaker flasks. Up and down scaling of T-DAB medium to 15 L bioreactor and 96 deep wells plate respectively, were successfully carried out as follows.

- T-DAB: Terrific-Defined Autoinduction Broth: 2.9 g/L glucose, 11.1 g/L glycerol, 7.6 g/L Lactose, 4 g/L (NH₄)₂HPO₄, 13.3 g/L KH₂PO₄, 1.6 g/L CaCl₂, 0.6 g/L MgSO₄·7H₂O, 0.1 g/L Fe(II) citrate, 2.1 mg/L Na₂MoO₄·2H₂O, 2.5 mg/L CoCl₂·6H₂O, 0.5 mg/L MnCl₂·4H₂O, 1.5 mg/L CuCl₂·2H₂O, 3 mg/L H₃BO₃, 53.8 mg/L ZnCl₂·(CH₃COO)₂·2H₂O, 14.1 mg/L Tryptix III, using NaOH or ammonia to bring final pH to 6.8.

- Influence of dissolved oxygen and temperature:

- **Yield & Solubility of target proteins**
  - **High**
  - **Low**

- **Temperature**
  - **High**
  - **Low**

- Better cultivation performance (yield and solubility of target proteins) are achieved at lower temperature and with cultivation vessel which can provide better oxygenation. In principal, using ammonia for pH adjustment of T-DAB medium to raise the nitrogen concentration also improves the yield of protein of interest.

- **Conclusion:** In conventional test tube and shaker flask, the autoinduction medium composition was successfully optimized.

To test the applicability of T-DAB medium to different *E. coli* T7 expression systems, expression of 8 recombinant proteins in three different *E. coli* strains (BL21 (DE3), Rosetta 2 (DE3) and BL21 CodonPlus (DE3) RIL) were carried out in 200 ml of T-DAB media in 1.8 L Fernbach flasks in comparison to IPTG induction using LB medium. The same expression levels were reached compared to conventional IPTG induced LB medium cultivation. The final (volumetric) yields obtained in autoinduction medium were about 4 times higher than during IPTG induction in LB medium.

**Advantage of autoinduction**

- Preculture
- Inoculation
- Harvest
- Induction
- Harvest

**Conventional induction method**

- Preculture
- Inoculation
- Harvest
- Induction
- Harvest

- **Legend to figure:** The target proteins are described in the first row of each SDS-PAGE. Growth medium and induction method are described in the second row. LB: *E. coli* grown in LB medium induced with 0.25 mM IPTG at 23°C. T-DAB: E. coli grown in T-DAB medium at 23°C. In the first row below each SDS-PAGE, W: Whole cellular protein; S: Soluble part of whole cellular protein; I: Insoluble part of whole cellular protein.

**Table:**

<table>
<thead>
<tr>
<th>Cultivation temperature (°C)</th>
<th>OD600</th>
<th>T-DAB medium</th>
<th>LB</th>
<th>T-DAB</th>
<th>LB</th>
<th>T-DAB</th>
<th>LB</th>
<th>T-DAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>0.25</td>
<td>20.0</td>
<td>6.0</td>
<td>20.5</td>
<td>5.4</td>
<td>19.5</td>
<td>7.6</td>
<td>27.4</td>
</tr>
</tbody>
</table>

**Figure:**

- Autoinduction medium optimization was carried out in test tubes and shaker flasks. Up and down scaling of T-DAB medium to 15 L bioreactor and 96 deep wells plate respectively, were successfully carried out as follows.

- T-DAB: Terrific-Defined Autoinduction Broth: 2.9 g/L glucose, 11.1 g/L glycerol, 7.6 g/L Lactose, 4 g/L (NH₄)₂HPO₄, 13.3 g/L KH₂PO₄, 1.6 g/L CaCl₂, 0.6 g/L MgSO₄·7H₂O, 0.1 g/L Fe(II) citrate, 2.1 mg/L Na₂MoO₄·2H₂O, 2.5 mg/L CoCl₂·6H₂O, 0.5 mg/L MnCl₂·4H₂O, 1.5 mg/L CuCl₂·2H₂O, 3 mg/L H₃BO₃, 53.8 mg/L ZnCl₂·(CH₃COO)₂·2H₂O, 14.1 mg/L Tryptix III, using NaOH or ammonia to bring final pH to 6.8.

- Influence of dissolved oxygen and temperature:

- **Yield & Solubility of target proteins**
  - **High**
  - **Low**

- **Temperature**
  - **High**
  - **Low**

- Better cultivation performance (yield and solubility of target proteins) are achieved at lower temperature and with cultivation vessel which can provide better oxygenation. In principal, using ammonia for pH adjustment of T-DAB medium to raise the nitrogen concentration also improves the yield of protein of interest.

- **Conclusion:** In conventional test tube and shaker flask, the autoinduction medium composition was successfully optimized.