

Additional file 4

Details of medium composition and preparation

Smart sustainable bottle (SSB) system for *E. coli* based recombinant protein production

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The composition of Luria-Bertani (**LB**) broth was as follows: 10 g L⁻¹ tryptone, 5 g L⁻¹ yeast extract, and 5 g L⁻¹ NaCl. The pH was adjusted at pH 7 by NaOH before autoclaving. For solidification, 15 g L⁻¹ agar was added.

The composition of Defined Non-inducing Broth (**DNB**) was as follows: 12 g L⁻¹ glucose.H₂O, 1.2 g L⁻¹ MgSO₄.7H₂O, 4 g L⁻¹ (NH₄)₂HPO₄, 13.3 g L⁻¹ KH₂PO₄, 1.7 g L⁻¹ citric acid.H₂O, 100.8 mg L⁻¹ Fe(III) citrate, 2.1 mg L⁻¹ Na₂MoO₄.2H₂O, 1.4 mg L⁻¹ CoCl₂, 12.3 mg L⁻¹ MnCl₂.2H₂O, 1.2 mg L⁻¹ CuCl₂, 3 mg L⁻¹ H₃BO₃, 33.8 mg L⁻¹ Zn(CH₃COOH)₂.2H₂O, 14.1 mg L⁻¹ Titriplex III. The pH was adjusted at pH 6.8 by NaOH.

The composition of “Smart”-Defined Autoinduction Broth (High Nitrogen Content) (**S-DAB (HNC)**) was as follows: 3.23 g L⁻¹ glucose.H₂O, 11.17 g L⁻¹ glycerol, 8 g L⁻¹ lactose.H₂O, 1.2 g L⁻¹ MgSO₄.7H₂O, 4 g L⁻¹ (NH₄)₂HPO₄, 13.3 g L⁻¹ KH₂PO₄, 1.7 g L⁻¹ citric acid.H₂O, 100.8 mg L⁻¹ Fe(III) citrate, 2.1 mg L⁻¹ Na₂MoO₄.2H₂O, 1.4 mg L⁻¹ CoCl₂, 12.3 mg L⁻¹ MnCl₂.2H₂O, 1.2 mg L⁻¹ CuCl₂, 3 mg L⁻¹ H₃BO₃, 33.8 mg L⁻¹ Zn(CH₃COOH)₂.2H₂O, 14.1 mg L⁻¹ Titriplex III. The pH was adjusted at pH 6.8 by ammonium hydroxide (~30%).

The composition of the DNB and S-DAB (HNC) media were described previously [1, 2]. To prevent precipitation during long-term storage, preparation methods were slightly modified as follows: the trace elements (CoCl₂, MnCl₂.2H₂O, CuCl₂, H₃BO₃, Zn(CH₃COOH)₂.2H₂O, and Titriplex III) were prepared as 2,000 times concentrated stock solution and stored at -20°C. Na₂MoO₄.2H₂O was prepared as 5,000 times concentrated stock solution and stored separately at -20°C. Carbon source(s) (glucose, etc.), MgSO₄ and 2,000 times trace elements stock solution were prepared together as a combined stock solution (A group) which was heat-sterilized (121°C, 20 min). Ammonium and phosphate salts additionally including citric acid, ferric citrate, and Na₂MoO₄.2H₂O were prepared as a combined stock solution (B group). After adjusting the pH to pH 6.8, the ammonium and phosphate salt stock solution (B group) can be heat-sterilized (121°C, 20 min).

References

1. Li Z, Kessler W, van den Heuvel J, Rinas U: **Simple defined autoinduction medium for high-level recombinant protein production using T7-based *Escherichia coli* expression systems.** *Appl Microbiol Biotechnol* 2011, 91:1203-1213.
2. Li Z, Nimitz M, Rinas U: **Optimized procedure to generate heavy isotope and selenomethionine-labeled proteins for structure determination using *Escherichia coli*-based expression systems.** *Appl Microbiol Biotechnol* 2011, 92:823-833

Table 1 2000 x trace element stock solution

Chemicals	mass
CoCl ₂ (MW: 129.84)	0.7 g
MnCl ₂ .2H ₂ O (MW: 161.87)	6.15 g
CuCl ₂ (MW: 134.45)	0.6 g
H ₃ BO ₃ (MW: 61.83)	1.5 g
Zn(CH ₃ COO) ₂ .2H ₂ O (MW: 219.49)	16.9 g
Titriplex III (EDTA-Na ₂ , MW: 372.24)	7.05 g

Bring the final volume to 0.25 L with double distilled water (ddH₂O), aliquot the solution and store at -20°C.

Table 2 5000 x Na₂MoO₄.H₂O stock solution

Chemicals	mass
Na ₂ MoO ₄ .2H ₂ O (MW: 241.95)	1.05 g

Bring the final volume to 0.1 L with double distilled water (ddH₂O), aliquot the solution and store at -20°C.

Table 3 DNB medium A group (0.5 L)

Chemicals and stock solution	mass & volume
glucose.H ₂ O (MW: 198.17)	60 g
MgSO ₄ .7H ₂ O (MW: 246.47)	6 g
2000 x trace element stock solution	2.5 mL

Bring the final volume to 0.5 L with double distilled water (ddH₂O) and autoclave at 121°C. Long term storage at room temperature is possible. The color of the solution may change without having negative effects.

Table 4 DNB medium B group (5 L)

Chemicals and stock solution	mass & volume
(NH ₄) ₂ HPO ₄ (MW: 132.06)	22.22 g
KH ₂ PO ₄ (MW: 136.09)	73.89 g
citric acid.H ₂ O (MW: 210.1)	9.44 g
Fe(III) citrate (MW: 244.94)	0.56 g
NaOH (MW: 40)	15 g
5000 x Na ₂ MoO ₄ .H ₂ O stock solution	1.11 mL

Add 4 L double distilled water (ddH₂O), use a hot plate magnetic stirrer for heating (about 60 to 80°C), and mix overnight to dissolve the Fe(III) citrate. After cooling to room temperature, adjust pH at pH 6.8. Bring the final volume to 5 L and autoclave at 121°C. Long time storage at room temperature is possible.

For preparation of 100 mL DNB medium, mix 10 mL A group, 90 mL B group and appropriate antibiotic in a clean bench.

Table 4 S-DAB medium A group (5 L)

Chemicals and stock solution	mass & volume
glucose.H ₂ O (MW: 198.17)	53.83 g
glycerol (MW: 92.09)	186.10 g
lactose.H ₂ O (MW: 360.31)	133.33 g
MgSO ₄ .7H ₂ O (MW: 246.47)	20.00 g
2000 x trace element stock solution	8.33 mL

Bring the final volume to 5 L with double distilled water (ddH₂O) and autoclave at 121°C. Long time storage at room temperature is possible. The color of the solution may change without having negative effects.

Table 5 S-DAB (HNC) medium B group (10 L)

Chemicals and stock solution	weight & volume
(NH ₄) ₂ HPO ₄ (MW: 132.06)	57.14 g
KH ₂ PO ₄ (MW: 136.09)	190 g
citric acid.H ₂ O (MW: 210.1)	24.29 g
Fe(III) citrate (MW: 244.94)	1.44 g
5000 x Na ₂ MoO ₄ .H ₂ O stock solution	2.86 mL

Add 8 L double distilled water (ddH₂O), use a hot plate magnetic stirrer for heating (about 60 to 80°C), and mix overnight to dissolve the Fe(III) citrate. After cooling to room temperature, adjust pH at pH 6.8 with appr. 55 ml ~30% ammonium hydroxide solution. Bring the final volume to 10 L and autoclave at 121°C. Long term storage at room temperature is possible.

For preparation of 1 L S-DAB (HNC) medium, mix 0.3 L A group, 0.7 L B group and appropriate antibiotic in a clean bench. For cultivation in SSB system or bioreactor, e.g. 2 L working volume, pour 1.4 L B group and 0.5 mL antifoam in SSB or bioreactor, prepare the cultivation vessel (e.g. calibration of pH and dissolved oxygen probes for the bioreactor) and autoclave at 121°C (previously prepared sterilized B group stock solution can be re-autoclaved). Mix appropriate antibiotic with 0.6 L A group in a sterilized container in a clean bench (re-autoclaving A group is not recommended). Aseptically transfer this mixture to the sterilized SSB system or bioreactor.

Table 6 “Booster” amino acids stock solution (125 mM for each amino acid, 1 L)

Chemicals	weight
L-arginine (MW: 174.2)	21.78 g
L-asparagine (MW: 132.12)	16.52 g
glycine (MW: 75.07)	9.38 g
L-proline (MW: 115.13)	14.39 g
L-leucine (MW: 131.17)	16.40 g
L-threonine (MW: 119.12)	14.89 g

Add 0.8 L double distilled water (ddH₂O) and use a magnetic stirrer to mix overnight. Then adjust pH at pH 7.5 and bring the final volume to 1 L. Sterilize by filtration (0.2 µm) and store at 4°C.