**Stock solutions**

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| **Stock solution** | **concentration, solvent** | **Note** |
| ammonium sulfate ((NH4)2SO4) | 1 M in ddH2O | sterilize by autoclaving |
| Amp100 | 100 mg/mL ampicillin in ddH2O |  |
| biotin | 10 g/L | sterilize by filtration through a 0.45 µm pore size syringe filter |
| calcium chloride (CaCl2) | 1 g/L | sterilize by filtration through a 0.45 µm pore size syringe filter |
| 3,4-dehydroproline | 50 mM in sterile NMMΔAA |  |
| 4,4-difluoroproline | 50 mM in sterile NMMΔAA |  |
| di-potassium hydrogen phosphate (K2HPO4) | 1 M in ddH2O | sterilize by autoclaving |
| DNase I | 1 mg/mL in ddH2O |  |
| (4*S*)-fluoroproline | 50 mM in sterile NMMΔAA |  |
| (4*R*)-fluoroproline | 50 mM in sterile NMMΔAA |  |
| *D*-glucose | 1 M in ddH2O | sterilize by filtration through a 0.45 µm pore size syringe filter |
| 20 % *D*-glucose | 200 g/L D-glucose in ddH2O | sterilize by filtration through a 0.45 µm pore size syringe filter |
| IPTG | 1 M in ddH2O |  |
| iron(II) chloride (FeCl2) | 1 g/L | sterilize by filtration through a 0.45 µm pore size syringe filter |
| lysozyme | 50 mg/mL in ddH2O |  |
| magnesium sulfate (MgSO4) | 1 M in ddH2O | sterilize by autoclaving |
| potassium dihydrogen phosphate (KH2PO4) | 1 M in ddH2O | sterilize by autoclaving |
| proline | 1 M in ddH2O |  |
| RNase A | 1 mg/mL in ddH2O |  |
| sodium-dodecylsulfate (SDS) | 200 g/L in ddH2O |  |
| sodium chloride (NaCl) | 5 M in ddH2O | sterilize by autoclaving |
| thiamine | 10 g/L | sterilize by filtration through a 0.45 µm pore size syringe filter |

**Trace elements mix**

Preparation:

1. Mix copper sulfate (CuSO4), zinc chloride (ZnCl2), manganese chloride (MnCl2), ammonium molybdate ((NH4)2MoO4); each 1 mg/L in ddH2O.
2. Sterilize by filtration through a 0.45 µm pore size syringe filter.

**20 (19) canonical aino acids mix**

Preparation:

1. Dissolve 0.5 g of L-phenylalanine and 0.5 g of L-tyrosine in 100 mL of ddH2O with dropwise addition of 1 M HCl under stirring until powder is dissolved.

2. Weigh out 0.5 g of each of the remaining *L*-amino acids (for **19** amino acids mix: **except *L*-proline**). Mix with 22 mL fo 1 M KH2PO4 and 48 mL of 1 M K2HPO4. Add ddH2O to about 800 mL. Stir until the solution becomes clear.

3. Add the dissolved *L*-phenylalanine and *L*-tyrosine from step 1 and adjust the volume to 1 L with ddH2O.

4. Sterilize the amino acid mixture by vacuum filtration with a bottle top filter unit.

**New minimal medium without canonical *L*-amino acids (NMMΔAA)**

1. Mix from the above stock solutions to obtain the following medium composition:

7.5 mM (NH4)2SO4

1.7 mM NaCl

22 mM KH2PO4

50 mM K2HPO4

1 mM MgSO4

20 mM *D*-glucose

1 µg/L CaCl2

1 µg/L FeCl2

10 µg/L thiamine

10 mg/L biotin

0.01 mg/L trace elements mix

**New minimal medium (NMM) with all 20 canonical *L*-amino acids**

1. Mix from the above stock solutions and add ddH2O to obtain the following medium composition:

7.5 mM (NH4)2SO4

1.7 mM NaCl

22 mM KH2PO4

50 mM K2HPO4

1 mM MgSO4

20 mM *D*-glucose

50 mg/L of **20** canonical amino acids mix

1 µg/L CaCl2

1 µg/L FeCl2

10 µg/L thiamine

10 mg/L biotin

0.01 mg/L trace elements mix

**New minimal medium containing all 19 canonical *L*-amino acids except *L*-proline (NMMΔPro)**

1. Mix from the above stock solutions and add ddH2O to obtain the following medium composition:

7.5 mM (NH4)2SO4

1.7 mM NaCl

22 mM KH2PO4

50 mM K2HPO4

1 mM MgSO4

20 mM *D*-glucose

50 mg/L of **19** canonical amino acids mix

1 µg/L CaCl2

1 µg/L FeCl2

10 µg/L thiamine

10 mg/L biotin

0.01 mg/L trace elements mix

**Phosphate-buffered saline (PBS)**

Composition: 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, 1 mM CaCl2, 0.5 mM MgCl2, pH 7, in ddH2O.

Preparation:

1. Dissolve appropriate amounts of the above salts for 1 L of medium in ~800 mL of ddH2O.

2. Adjust pH to a value of 7.

3. Fill up volume to 1 L.

4. Sterilize by autoclaving or filtration through a 0.45 µm pore size filter.

**Binding buffer**

Composition: 50 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, pH 8, in ddH2O

Preparation:

1. Dissolve appropriate amounts of the above substances for 1 L of buffer in ~800 mL of ddH2O.

2. Adjust pH to a value of 8.

3. Fill up volume to 1 L.

**Dialysis buffer**

Composition: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4∙7H2O, 1.4 mM KH2PO4, pH 7.3, in ddH2O

Preparation:

1. Dissolve appropriate amounts of the above substances for 1 L of buffer in ~800 mL of ddH2O.

2. Adjust pH to a value of 7.3.

3. Fill up volume to 1 L.

**Elution buffer**

Composition: 50 mM Tris-HCl, 150 mM NaCl, 200 mM imidazole, 1 mM DTT, pH 8, in ddH2O

Preparation:

1. Dissolve appropriate amounts of the above substances for 1 L of buffer in ~800 mL of ddH2O.

2. Adjust pH to a value of 8.

3. Fill up volume to 1 L.

**Wash buffer**

Composition: 50 mM Tris-HCl, 500 mM NaCl, 10 mM imidazole, 1 mM DTT, pH 8, in ddH2O

Preparation:

1. Dissolve appropriate amounts of the above substances for 1 L of buffer in ~800 mL of ddH2O.

2. Adjust pH to a value of 8.

3. Fill up volume to 1 L.

**5x SDS loading dye buffer**

Composition: 0.25 M Tris pH 6.8, 50% v/v glycerol, 0.25% w/v bromphenol blue, 0.5 M dithiothreitol (DTT; alternatively 5% β-mercaptoethanol), 10% w/v sodium-dodecylsulfate (SDS) in ddH2O

Preparation:

1. Dissolve appropriate amounts of the above substances for 10 mL of buffer in ~8 mL of ddH2O.

3. Fill up volume to 10 mL.

**MS buffer**

Composition: 10 mM Tris-HCl, pH 8, in ddH2O

1. Dissolve appropriate amount of Tris-HCl for 1 L of buffer in ~800 mL of ddH2O.

2. Adjust pH to a value of 8.

3. Fill up volume to 1 L.

**Lysogeny Broth (LB) medium**

Composition: 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, pH 7.0 in ddH2O

Preparation:

1. Weigh out 50 g of tryptone, 25 g of yeast extract, 5 g of NaCl into a 1 L glass bottle.

2. Add ddH2O up to ~800 mL and dissolve components under stirring.

3. Measure the pH and adjust to pH 7 by dropwise addition of 1 M HCl or 1 M NaOH, if necessary. Add ddH2O up to 1 L.

4. Sterilize by autoclaving, check for volume loss afterwards and add sterile ddH2O to compensate if necessary.

5. Store at 4 °C until use.

**Luria Agar (LA) medium plates**

Composition: 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, 15 g/L agar-agar, pH 7.0 in ddH2O

Preparation:

1. Weigh out 50 g of tryptone, 25 g of yeast extract, 5 g of NaCl, 7.5 g of agar-agar into a 1 L glass bottle.

2. Add ddH2O up to 500 mL and dissolve components under stirring.

3. Measure the pH and adjust to pH 7 by dropwise addition of 1 M HCl or 1 M NaOH, if necessary. Add ddH2O up to 1 L.

4. Sterilize by autoclaving, check for volume loss afterwards and add sterile ddH2O to compensate, if necessary. (Note: LB agar can be stored at 4 °C until use for preparation of LB agar plates. Carefully melt solidified agar using a microwave)

5. When the solution is still warm (30–40 °C), add ampicillin to a final concentration of 100 µg/mL

6. Pour about 15 mL of the liquid from step 5 into a sterile 10 cm Petri dish under sterile conditions. When the agar is solidified, plates can be stored for 1 week at 4 °C until use.