

New Media Recipes (09/01/2006)
Center for Structural Biology of Membrane Proteins

Modification of Recipes from: *Studier FW: Protein production by auto-induction in high density shaking cultures. Protein Expr Purif 2005, 41:207-234.*

A1. LBE (enhanced) media – To make 1 liter:

5 g	yeast extract
10 g	tryptone (or peptone)
5 g	glucose
10 g	NaCl

Add distilled water to the reagents to make 900 mL in a measuring cylinder. Mix until all material is dissolved. Autoclave, then add:

1. 1.0 mL sterile 2.0 M MgSO₄
2. 1.0 mL metals mix
3. 100 mL filter sterilized potassium phosphate mix (for 100 mM phosphate):

Component

1 M KH ₂ PO ₄	20 mL
1 M K ₂ HPO ₄	80 mL

A2. LBE-LP (low phosphate) media – To make 1 liter:

5 g	yeast extract
10 g	tryptone (or peptone)
1 g	glucose
5 g	NaCl

Add distilled water to the reagents to make 900 mL in a measuring cylinder. Mix until all material is dissolved. Autoclave, then add:

1. 1.0 mL sterile 2.0 M MgSO₄
2. 1.0 mL metals mix
3. 100 mL filter sterilized potassium phosphate mix (for 25 mM phosphate):

Component

1 M KH ₂ PO ₄	5 mL
1 M K ₂ HPO ₄	20 mL
dH ₂ O	75 mL

A3. LBE-505 media – To make 1 liter

5 g	yeast extract
10 g	tryptone (or peptone)
5 g	glycerol
0.5g	glucose
0.7 g	Na ₂ SO ₄
2.5 g	NH ₄ Cl

Add distilled water to the reagents to make 900 mL in a measuring cylinder. Mix until all material is dissolved. Autoclave, then add:

1. 1.0 mL sterile 2.0 M MgSO₄
2. 1.0 mL metals mix
3. 100 mL filter sterilized potassium phosphate mix (for 50 mM phosphate):

Component

1 M KH ₂ PO ₄	10 mL
1 M K ₂ HPO ₄	40 mL
dH ₂ O	50 mL

A4. LBE-5052 autoinducing media - to make 1 liter:

5 g	yeast extract
10 g	tryptone (or peptone)
5 g	glycerol
0.5g	glucose
2 g	lactose
0.7 g	Na ₂ SO ₄
2.5 g	NH ₄ Cl

Add distilled water to the reagents to make 900 mL in a measuring cylinder. Mix until all material is dissolved. Autoclave, then add:

1. 1.0 mL sterile 2.0 M MgSO₄
2. 1.0 mL metals mix
3. 100 mL filter sterilized potassium phosphate mix (for 50 mM phosphate):

Component

1 M KH ₂ PO ₄	10 mL
1 M K ₂ HPO ₄	40 mL
dH ₂ O	50 mL

B. 2xTYE (enhanced) media To make 1 liter

10 g	yeast extract
16 g	tryptone (or peptone)
5 g	glucose
5 g	NaCl

Add distilled water to the reagents to make 900 mL in a measuring cylinder. Mix until all material is dissolved. Autoclave, then add:

1. 1.0 mL sterile 2.0 M MgSO_4
2. 1.0 mL metals mix
3. 100 mL sterile potassium phosphate mix (for 100 mM phosphate):

Component

1 M KH_2PO_4	20 mL
1 M K_2HPO_4	80 mL

C. Terrific Broth “Enhanced” To make 1 liter

Bacto-tryptone	12g
Bacto-yeast extract	24g
Glycerol	4ml

Add distilled water to the reagents to make 900 ml in a measuring cylinder. Mix until all material is dissolved. Autoclave, then add:

1. 1.0 mL sterile 2.0 M MgSO_4
2. 1.0 mL sterile metals mix
3. 100 mL sterile potassium phosphate mix (for 100 mM phosphate):

Component

1 M KH_2PO_4	20 mL
1 M K_2HPO_4	80 mL

1000x metals mix (100 ml in ~50 mM HCl)

1. Make 50 ml of 0.1 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (FW: 270.30) by dissolving the salt in ~0.1 M HCl (a 100-fold dilution of conc HCl).

2. Add together:

1 ml 1 M $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (FW: 197.91)

1 ml 1 M $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (FW: 287.56)

1 ml 0.2 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (FW: 237.95)

1 ml 0.2 M $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (FW: 237.72)

46 ml distilled water

Filter sterilize

Mix the FeCl_3 stock (50 mL) with the sterilized metals solution (50 mL) to make a 100 mL of **1000x metals mix** in ~50 mM HCl. The final concentrations in 1000-fold dilution are: 50 μM Fe, 10 μM Mn, 10 μM Zn, 2 μM Ni, 2 μM Co. This solution was stored at room temperature. Upon prolonged storage, small amounts of precipitate formed in the mixture.