Growth Media

Tuesday, March 27, 2012
2:20 PM

LB Liquid
200 mL dH2O
+5g LB broth (Acumedia #7279)
Autoclave for 25 minutes

LB Plates
200 mL dH20
+5g LB broth (Acumedia #7279)
+3g Agarose (Invitrogen #16500-500)
Autoclave for 25 minutes and allow to cool to touch. Add appropriate amount of antibiotics and pour ~35mL/plate in a sterile workspace. Store at 4°C

MM3 Media for Batch Cultures

Adjust Glucose and yeast as needed
Seed culture we typically use 5 g/L glucose, 5g/L yeast extract
Experimental culture we typically use 5 g/L glucose, 1 g/L yeast extract

Components	1 L	250 ml	150 ml	
dH_2O	600 ml	150 ml	90 ml	
50% Glucose (5 g L^{-1}, 27.8 mM batch)	8.2 ml	2.05 ml	1.25 ml	
1 M MOPS	100 ml	25 ml	15 ml	
10X Medium concentrate	100 ml	25 ml	15 ml	
10X Phosphate buffer	100 ml	25 ml	15 ml	
20X $CaCl_2$	50 ml	12.5 ml	7.5 ml	
100X Trace Elements	10 ml	2.5 ml	1.5 ml	
1 mg/ml Thiamine (2 mg L^{-1} batch)	2 ml	0.5 ml	0.3 ml	
100 g/L Yeast extract (1 g L^{-1} batch)	10 ml	2.5 ml	1.5 ml	

pH to 7.0 using NaOH or HCl (usually not necessary)
Adjust volume to final volume
Filter sterilize and store at 4°C

Concentrated Solutions

50% Glucose Solution 250 mL
In 250mL dH_2O
+ 125 g Glucose (fw 180) 5.76 M stock, 94.5 mM batch
Filter sterilize or autoclave

Yeast Extract 100 g/L
In 150 ml

+ 15 g of yeast extract
Filter sterilize

1 mg/ml Thimine Solution
In 50 ml H_2O
+ 0.05 g Thiamine HCl
Filter sterilize and store at 4°C

Medium Concentrate 10x
Dissolve following in 850mL dH_2O:
+ 50 g $(NH_4)_2SO_4$, (fw 132.1) 380 mM stock, 38 mM batch
+ 5 g $MgSO_4$ (fw 120.3) 41.5 mM stock, 4 mM batch
Adjust to 1L after dissolved for final volume

Phosphate Buffer 10x
Dissolve following in 1 L dH_2O:
+ 4 g KH_2PO_4 (fw 136.09) 29 mM stock, 2.9 mM batch
+10 g Na_2HPO_4 (fw 141.96) 71 mM stock, 7.1 mM batch
Adjust to pH of 7 with NaOH or HCl

Calcium chloride Solution 20x
In 1L dH_2O
+ 2 g $CaCl_2$ (fw 147) 13.5 mM stock, 0.7 mM batch

MOPS Buffer 1M
In 600 ml dH_2O
+ 209.3 g MOPS (fw 209.26) 1 M stock, 100 mM batch
Adjust to 1 L after adding MOPS for final volume
Adjust to pH 7.0 with NaOH or HCl

Trace Elements Solution 100x
NOTE: The weight for chemicals with different hydration levels should be adjusted on molecular weight base.
In 1L of dH_2O
+10 g Citric acid monohydrate (fw 210.14) 47.5 mM stock, 475 µM batch
+2 g $MnSO_4*2H_2O$ (fw 183)* 10.9 mM stock, 109 µM batch
+0.5 g $FeSO_4*7H_2O$ (fw 277.9) 1.8 mM stock, 18 µM batch
+0.2 g $ZnSO_4*7H_2O$ (fw 280.54) 710 µM stock, 7.1 µM batch
+ 0.05 g $CuSO_4*5H_2O$ (fw 249.6) 200 µM stock, 2 µM batch
+0.05 g $Na_2MoO_4*2H_2O$ (fw 241.95) 207 µM stock, 2.1 µM batch
+ 0.05 g $CoCl_2*6H_2O$ (fw 237.93) 210 µM stock, 2.1 µM batch
Filter sterilize and store at 4°C

MM1 for Batch Cultures

Materials:
Glucose
$(NH_4)_2SO_4$
$MgSO_4$
KH_2PO_4
Na_2PO_4

$CaCl_2*2H_2O$
Citric acid monohydrate
$MnSO_4*2H_2O$
$FeSO_4*7H_2O$
$ZnSO_4*7H_2O$
$CuSO_4*5H_2O$
$NaMoO*2H_2O$
Yeast extract
Thiamine
Filter sterilization flasks, 1 L and 150 ml

Stocks:

50% Glucose Solution, 250 mL
In 250mL dH_2O
+ 125 g Glucose (fw 180) 5.76 M stock, 94.5 mM batch
Filter sterilize or autoclave

10X Medium concentrate, 1L
Dissolve following in 850mL dH_2O:
+ 50 g $(NH_4)_2SO_4$, (fw 132.1) 380 mM stock, 38 mM batch
+ 10 g $MgSO_4$ (fw 120.3) 83 mM stock, 8 mM batch
Adjust to 1L after dissolved for final volume
Autoclave

10X Phosphate Buffer, 1 L
Dissolve following in 850mL dH_2O:
+ 50 g KH_2PO_4 (fw 136.09) 367 mM stock, 36.7 mM batch
+120 g Na_2HPO_4 (fw 140.95) 850 mM stock, 85 mM batch
Titrate to a pH of 7 with 5N NaOH or H_2SO_4
*NOTE: The Na_2HPO_4 takes a long time to dissolve, add to solution in 30 g increments
Autoclave

20X $CaCl_2$ Solution, 1 L
In 1L dH_2O
+ 4 g $CaCl_2$ (fw 147) 27 mM stock, 1.36 mM batch
Autoclave

MM1 Media for Batch Cultures Methods:

100X Trace Elements Solution, 1 L
NOTE: The weight for chemicals with different hydration levels should be adjusted on molecular weight base.
In 1L of dH_2O
+10 g Citric acid monohydrate (fw 210.14) 47.5 mM stock, 475 μM batch
+2 g $MnSO_4*2H_2O$ (fw 183)* 10.9 mM stock, 109 μM batch
+0.5 g $FeSO_4*7H_2O$ (fw 277.9) 1.8 mM stock, 18 μM batch
+0.2 g $ZnSO_4*7H_2O$ (fw 280.54) 710 μM stock, 7.1 μM batch
+ 0.02 g $CuSO_4*5H_2O$ (fw 249.6) stock, mM batch
+0.02 g $Na_2MoO_4*2H_2O$ (fw 241.95) 82.6 μM stock, 0.826 μM batch
Autoclave

100 g/L Yeast Extract
In 150 ml
+ 15 g of yeast extract
Filter sterilize

1 mg/ml Thimine Solution
In 50 ml H_2O
+ 0.05 g Thiamine HCl
Filter sterilize and store at 4°C

MM1 Media for Batch Cultures

Components	1 L	250 ml	150 ml	
dH_2O	600 ml	150 ml	90 ml	
50% Glucose (10 g L^{-1}, 55.5 mM batch)	16.4 ml	4.1 ml	2.5 ml	
10X Medium concentrate	100 ml	25 ml	15 ml	
10X Phosphate buffer	100 ml	25 ml	15 ml	
20X $CaCl_2$	50 ml	12.5 ml	7.5 ml	
100X Trace Elements	10 ml	2.5 ml	1.5 ml	
1 mg/ml Thiamine (2 mg L^{-1} batch)	2 ml	0.5 ml	0.3 ml	
100 g/L Yeast extract (1 g L^{-1} batch)	10 ml	2.5 ml	1.5 ml	

pH to 7.0 using NaOH or HCl
Adjust volume to final volume
Filter sterilize and store at 4°C