

E. coli Genome Project

University of Wisconsin - Madison



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RESOURCES

EZ Rich Defined Medium

Updated 5/5/03. [Now this medium is available commercially.](#)

Updated 6/14/02. This is our protocol for preparing Neidhardt Supplemented MOPS Defined Medium. It is called "EZ" because modifications were made to the details of the protocol from Neidhardt's lab to streamline it (i.e. Neidhardt's lab recipe called for fresh cysteine, but we mix the cysteine with the other amino acids in the frozen supplement solution) and to correct some minor errors.

Reference: F. C. Neidhardt, P. L. Bloch, and D. F. Smith. 1974. Culture medium for enterobacteria. *J Bacteriol* 119(3): 736-747 [[PubMed Central](#)].

EZ Rich Defined Medium (EZ RDM)

10X MOPS Mixture	100ml
0.132 M K ₂ HPO ₄	10 ml
10X ACGU	100 ml
5X Supplement EZ	200 ml
sterile H ₂ O	580 ml
TOTAL	990 ml

Before use add 100X carbon source (as specified): 10 ml

EZ Rich Defined Medium Plates (EZ RDM Plates)

10X MOPS Mixture	100 ml
0.132 M K ₂ HPO ₄	10 ml
10X ACGU	100 ml
5X Supplement EZ	200 ml
sterile H ₂ O	80 ml
TOTAL	490 ml

In a separate 2 L flask, mix 13 g Bacto Agar and 500 ml of water. Autoclave 20 minutes on liquid cycle. Add the mixture above to the flask within about 15 minutes of removing it from the autoclave. Add 10 mL of 100X carbon source (usually specified, but if not, use 20% glucose) and antibiotics (if specified). Pour immediately.

10X MOPS mixture

1. In a 1 L beaker with a stir bar, add the following to ~300 ml milliQ H₂O:

Component	FW	grams
MOPS	209.3	83.72
Tricine	179.2	7.17

2. Add 10 M KOH to a final pH of 7.4 (10 to 20 ml)
3. Bring total volume to 440 ml
4. Make fresh FeSO₄ solution and add it to the MOPS/Tricine solution:

Component	FW	grams	H ₂ O vol (ml)	stock conc (M)
FeSO ₄ •7H ₂ O	278	0.028	10	0.01

5. Add the following solutions to the MOPS/Tricine/FeSO₄ solution (see below how to make each of these):

Mix in the order shown!

Component	Volume
1.9 M NH ₄ Cl	50 ml
0.276 M K ₂ SO ₄	10 ml
0.02 M CaCl ₂ •2H ₂ O	0.25 ml
2.5 M MgCl ₂	2.1 ml
5 M NaCl	100 ml
Micronutrient stock	0.2 ml
Autoclaved milliQ H ₂ O	387 ml
TOTAL	1000 ml

6. Filter sterilize with 1 L capacity 0.2 micron filter

7. Aliquot into sterile 100 or 200 ml plastic bottles and freeze at -20 degrees.

Stocks used in 10X MOPS mixture

Make each separately, mixing the amount indicated into the specified volume. Store at room temp.

Component	FW	stock conc (M)	grams	vol (ml)	note
NH ₄ Cl	53.49	1.9	50.82	500	
K ₂ SO ₄	174.3	0.276	4.8	100	
CaCl ₂ •2H ₂ O	147	0.02	0.294	100	
MgCl ₂	203.3	2.5	50.75	100	
NaCl	58.44	5	292.2	1000	

Micronutrient stock (50 ml)

Mix everything together in 40 ml autoclaved MQ-H₂O, bring up total volume to 50 ml. Store at room temp.

Component	Formula	FW	Grams for 50 ml
ammonium molybdate	(NH ₄) ₆ Mo ₇ O ₂₄ •4H ₂ O	1235.9	0.009
boric acid	H ₃ BO ₃	61.83	0.062
cobalt chloride	CoCl ₂	237.9	0.018
cupric sulfate	CuSO ₄	249.7	0.006
manganese chloride	MnCl ₂	197.9	0.040
zinc sulfate	ZnSO ₄	287.5	0.007

Potassium phosphate K₂HPO₄ Solution

NOTE!! Make sure you use dibasic K₂HPO₄, and do NOT use monobasic KH₂PO₄ !!!!! Can be stored at room temperature after autoclaving.

Component	FW	stock conc. (M)	grams	Vol (ml)	note
K ₂ HPO ₄	173.2	0.132	23.0	1000	Autoclave

10X ACUG (1000 ml)

1. In a 1 L beaker with stir bar, add 1.5 ml 10 M KOH to 1 L milliQ H₂O to make 0.015 M KOH

2. Dissolve the following together:

Component	FW	Grams for 1000 ml	Note
adenine	135.13	0.270	Dissolve together in 0.015 M KOH
cytosine	111.1	0.222	Filter sterilize
uracil	112.09	0.224	Store at -20° C
guanine	151.13	0.302	

3. Filter sterilize with 1 L capacity 0.2 micron filter

4. Aliquot into sterile 100 or 200 ml plastic bottles and freeze at -20° C .

5X Supplement EZ (1000 ml)

Mix together the following solutions (see below for how to make each). Filter sterilize with 1 L capacity 0.2 micron filter. Aliquot into sterile 100 or 200 ml plastic bottles and freeze at -20° C.

Stock solution	Volume (ml)
alanine (free)	5
arginine (HCl)	65
asparagine (free)	40
aspartic acid (K salt)	5
cysteine (HCl H ₂ O)	50
glutamic acid (K salt)	5
glutamine (free)	25
glycine (free)	5
histidine (HCl H ₂ O)	5
isoleucine (free)	10
leucine (free)	80
lysine	5
methionine (free)	5
phenylalanine (free)	40
proline (free)	5
serine (free)	125
threonine (free)	5
tryptophane (free)	10
tyrosine (free)	100
valine (free)	10
VA Vitamin Solution	50
H ₂ O	350
TOTAL	1000

Amino acid stocks used in 5X Supplement

Make each separately, mixing the amount indicated into the specified volume. Store at -20° C.

Name	FW	Weight (g)	Vol (ml)	Notes
alanine (free)	89.09	1.78	25	
arginine (HCl)	210.70	8.44	100	
asparagine (free)	132.10	0.66	100	
aspartic acid (K salt)	171.20	1.71	25	
cysteine (HCl H ₂ O)	175.60	0.09	50	will oxidize to cystine, so 5X supplement and RDM should be frozen
glutamic acid (K salt)	185.30	2.78	25	

glutamine (free)	146.20	1.8	100	
glycine (free)	75.07	1.50	25	
histidine (HCl H ₂ O)	209.60	1.05	25	
isoleucine (free)	131.20	0.65	25	
leucine (free)	131.20	0.66	100	
lysine	182.70	1.83	25	
methionine (free)	149.20	0.75	25	
phenylalanine (free)	165.20	0.83	100	
proline (free)	115.10	1.15	25	
serine (free)	105.10	4.20	100	
threonine (free)	119.10	1.19	25	
tryptophane (free)	204.20	0.26	25	
tyrosine (free)	181.20	0.045	25	dissolve tyrosine in 0.01 M KOH
valine (free)	117.20	0.88	25	

100X VA Vitamin Solution (500 ml)

Mix together the following solutions (see below for how to make each): Store at -20° C.

Stock solution	volume
0.02 M thiamine HCl	25
0.02 M calcium pantothenate	25
0.02 M <i>p</i> -aminobenzoic acid	25
0.02 M <i>p</i> -hydroxybenzoic acid	25
0.02 M 2,3-dihydroxybenzoic acid	25
milliQ H ₂ O	375
TOTAL	500

Stock solutions used in 100X VA

Make each separately, mixing the amount indicated into the specified volume

Name	FW	grams	volume (ml)	
thiamine HCl	337.3	0.169	25	
calcium pantothenate	476.5	0.238	25	
<i>p</i> -aminobenzoic acid	137.13	0.069	25	dissolve in 0.02 M KOH
<i>p</i> -hydroxybenzoic acid	138.13	0.069	25	dissolve in 0.02 M KOH
2,3-dihydroxybenzoic acid	154.12	0.077	25	dissolve in 0.02 M KOH

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