

A Rich Defined Medium for SeMet labelling with non-deficient strains

Modified from: <http://www.genome.wisc.edu/resources/protocols/ezmedium.htm>, based on Neidhardt's MOPS medium.

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

Protocol for SeMet labelling.

1. Transform and plate out BL21 (plysS etc.) on EZC-Met Agar with appropriate Antibiotics.
2. Incubate ON at 37°C.
3. Transfer bacteria from plate into prewarmed EZC-Met complete medium with appropriate antibiotics.
4. Prewarm main culture medium (e.g. 12l).
5. Grow preculture (100 – 500ml) until OD₆₀₀ of 0.5 is reached.
6. Incubate prewarmed main culture medium containing antibiotics and carbon source. A t_a of 40 min can be expected for BL21(DE3)plysS.
7. At OD₆₀₀ of ca. 0.3 add 40 g/l SeMet.
8. If applicable, reduce temperature according to your expression protocol.
9. Induce at your usual OD₆₀₀ (e.g. 0.8).

EZC Rich Defined Medium (EZC RDM)

10X MOPS Mixture	100ml
0.132 M K ₂ HPO ₄	10 ml
10X ACGU	100 ml
10X Supplement EZC	100 ml
sterile H ₂ O	680 ml
TOTAL	990 ml

Before use add 100X carbon source (40% glucose): 10 ml

EZC Rich Defined Medium Plates (EZC 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	
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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 .

10X Supplement EZC (1000 ml)

Dissolve first Tyrosine in 200ml 10mM KOH. Fill up to 950l and dissolve the rest. Solution can be heated to 50°C for faster dissolution.

Add Vitamine solution.

Amino Acid	[mg]
alanine (free)	50
arginine (HCl)	1000
asparagine (free)	50
aspartic acid (K salt)	50

cysteine (HCl H ₂ O)	50
glutamic acid (K salt)	100
glutamine (free)	100
glycine (free)	50
histidine (HCl H ₂ O)	50
isoleucine (free)	10
leucine (free)	50
lysine	100
methionine (free)	No !!!
phenylalanine (free)	100
proline (free)	50
serine (free)	1000
threonine	100
tryptophane (free)	20
tyrosine (free)	50
valine (free)	100
VA Vitamin Solution	50 ml
H ₂ O ad	1000

200X VA Vitamin Solution (500 ml)

Mix the following compounds, store at -20° C.

Stock solution	grams	
0.02 M <i>p</i> -aminobenzoic acid	0.069	(dissolve in 40ml 0.01 M KOH)
0.02 M <i>p</i> -hydroxybenzoic acid	0.069	(dissolve in 40ml 0.01 M KOH)
0.02 M 2,3-dihydroxybenzoic acid	0.077	(dissolve in 40ml 0.01 M KOH)
0.02 M thiamine HCl	0.169	
0.02 M calcium pantothenate	0.238	
TOTAL	250 ml	