

# E. coli Genome Project

University of Wisconsin - Madison



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## RESOURCES

### MOPS Minimal Medium

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

Updated 6/14/02. Our protocol for preparing Neidhardt MOPS Minimal Medium.

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

#### MOPS Minimal Medium

10X MOPS mixture	100 ml	
0.132 M K <sub>2</sub> HPO <sub>4</sub>	10 ml	
milliQ H <sub>2</sub> O	880 ml	
1mg/ml thiamine	0.1 ml	(optional - we do not use thiamine because it does not affect the growth rate of <i>E. coli</i> K-12 MG1655 in this medium.)
TOTAL	990 ml	

- Mix ingredients above and adjust the pH to 7.2 with approximately 300 microliters 10 M NaOH.
- Filter sterilize. Can be stored at 4 degrees for up to 1 month.
- Before use add 10 ml 100X carbon source (as appropriate - we typically use a final concentration of 0.1% glucose).

#### 10X MOPS Mixture

- In a 1 L beaker with a stir bar, add the following to ~300 ml milliQ H<sub>2</sub>O:

Component	FW	grams
MOPS	209.3	83.72
Tricine	179.2	7.17

- Add 10 M KOH to a final pH of 7.4 (10 to 20 ml)
- Bring total volume to 440 ml
- Make fresh FeSO<sub>4</sub> solution and add it to the MOPS/Tricine solution:

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

- Add the following solutions to the MOPS/tricine/FeSO<sub>4</sub> solution (see below how to make each of these):

Mix in the order shown!

Component	Volume
1.9 M NH <sub>4</sub> Cl	50 ml
0.276 M K <sub>2</sub> SO <sub>4</sub>	10 ml
0.02 M CaCl <sub>2</sub> •2H <sub>2</sub> O	0.25 ml

2.5 M MgCl <sub>2</sub>	2.1 ml
5 M NaCl	100 ml
Micronutrient stock	0.2 ml
Autoclaved milliQ H <sub>2</sub> 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°.

#### *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 <sub>4</sub> Cl	53.49	1.9	50.82	500	
K <sub>2</sub> SO <sub>4</sub>	174.3	0.276	4.8	100	
CaCl <sub>2</sub> •2H <sub>2</sub> O	147	0.02	0.294	100	
MgCl <sub>2</sub>	203.3	2.5	50.75	100	
NaCl	58.44	5	292.2	1000	

#### *Micronutrient stock (100 ml)*

Mix everything together in 40 ml autoclaved milliQ H<sub>2</sub>O, bring up total volume to 50 ml. Store at room temp.

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

#### *Potassium phosphate K<sub>2</sub>HPO<sub>4</sub> Solution:*

NOTE!! Make sure you use dibasic K<sub>2</sub>HPO<sub>4</sub>, and do NOT use monobasic KH<sub>2</sub>PO<sub>4</sub> !!!!! Can be stored at room temperature after autoclaving.

Component	FW	stock conc (M)	grams	Vol (ml)	note
K <sub>2</sub> HPO <sub>4</sub>	173.2	0.132	23.0	1000	autoclave

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