

# Zhang-Mills-Block 1 (ZMB1) Media

**ZMB1 media consists of 22 chemical groups. The following is the protocol to make the 22 stock solutions for these groups followed by the protocol to mix the stock solutions to make the final ZMB1 media.**

- the concentrations of the chemical components were referenced from Table S1 in "Development of Chemically-Defined Media Supporting High Cell Density Growth of Lactococci, Enterococci, and Streptococci"- Zhang, G. Appl Environ Microbiol. 2009
- Syringe-filter sterilization (SFS) was performed using 0.22um, pore size, Millex GP filter unit from Millipore.
- the sugar component, Grp1, was excluded from the protocol and a couple chemicals were reorganized into their own chemical groups.
- all stock solutions were transferred to 50 mL falcon tubes before storage at -20°C
- 125 mL flasks were used to dissolve the chemicals prior to transferring to 50 mL falcon tubes
- all stock solutions were 50 mL except for Grp8, Vitamin: myo-inositol, which was 100 mL

## **1. Grp2- EAA#1: L-Histidine (5 g/L)**

- I. Add 250mg of L-Histidine to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to flask; continued stirring (CS) until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IV. Store at -20°C

## **2. Grp3- EAA#2: L-Isoleucine (20 g/L)**

- I. Add 1g of L-Isoleucine to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to flask; CS until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IV. Store at -20°C

## **3. Grp4- EAA#3: L-Leucine (25 g/L)**

- I. Add 1.25g of L-Leucine to flask with stir-bar
- II. Add 10 mL of H<sub>2</sub>O to flask.
- III. Add 5 mL of 1N NaOH; CS until dissolved.
- IV. Raise volume, with H<sub>2</sub>O, to 50 mL.
- V. Store at -20°C

## **4. Grp5- EAA#4: L-Methionine (15 g/L)**

- I. Add 750mg of L-Methionine to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to flask; CS until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IV. Store at -20°C

## **5. Grp6- EAA#5: L-Valine (17.5 g/L)**

- I. Add 875mg of L-Valine to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to flask; CS until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IV. Store at -20°C

## **6. Grp7- EAA#6: L-Arginine (20 g/L)**

- I. Add 1g of L-Arginine to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to flask; CS until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL
- IV. Store at -20°C

## **7. Grp8- Vitamin: myo-Inositol (0.1 g/L)**

- I. Add 10mg of myo-Inositol to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to flask; CS until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 100 mL.
- IV. Store at -20°C

## **8. Grp9- Phosphate buffers: KH<sub>2</sub>PO<sub>4</sub> (52.4 g/L), K<sub>2</sub>HPO<sub>4</sub> (107.1 g/L)**

- I. Add 2.6g of KH<sub>2</sub>PO<sub>4</sub> and 5.4g of K<sub>2</sub>HPO<sub>4</sub> to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to bottle and Stir (by hand) until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IV. Store at -20°C

## **9. Grp10- Other amino acid group: L-Glutamic acid (15 g/L), L-Phenylalanine (10 g/L), L- Proline (17.5 g/L), L-Asparagine (12.5 g/L), L-Aspartic acid (1.25 g/L), L-Glutamine (15 g/L), L-Serine (12.5 g/L), L-Threonine (12.5 g/L), L-Cysteine HCl (5 g/L), L-Alanine (10 g/L), Glycine (7.5 g/L), L-Lysine HCl (12.5 g/L), L-Tryptophan (5 g/L)**

- I. Add 750mg of L-Glutamine to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to bottle; CS until dissolved.
- III. Add 500mg of L-Phenylalanine to flask and Stir. There will be some crystals, but they will dissolve eventually; CS.
- IV. Add 875mg of L-Proline; (CS)

## Zhang-Mills-Block 1 (ZMB1) Media

- V. Add 625mg of L-Serine; CS
- VI. Add 625mg of L-Threonine; CS
- VII. Add 250mg of L-Cysteine. CS
- VIII. Add 500mg of L-Alanine; CS
- IX. Add 375mg of Glycine; CS
- X. Add 625mg of L-Lysine HCl; CS
- XI. Add 750mg of L-Glutamic Acid. This will not dissolve in water.
- XII. Slowly add 8 mL of 1N NaOH, drop by drop. Also used this volume of NaOH to wash the sides of the flask; CS
- XIII. Add 625mg of L-Asparagine; CS
- XIV. Add 62.5mg of L-Aspartic acid; CS
- XV. Add 250mg of L-Tryptophan; CS until dissolved.
- XVI. Raise volume, with H<sub>2</sub>O, to 50 mL.
- XVII. Store at -20°C

### 10. Grp11- Important Vitamin group: Calcium pantothenate (0.1 g/L), Niacin (0.075 g/L), Pyridoxal HCl (0.4 g/L)

- I. Make (1.0 g/L) solution of Calcium pantothenate by initially adding 50mg of Calcium pantothenate and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- II. Make (0.75 g/L) solution of Niacin by initially adding 37.5mg of Niacin and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- III. In a new 125 mL flask with stir-bar, Add 20mg of Pyridoxal HCl.
- IV. Add 5 mL of (1.0 g/L) Calcium pantothenate solution and 5 mL of (0.75 g/L) Niacin solution.
- V. Add 20 mL of H<sub>2</sub>O to flask; CS until dissolved.
- VI. Raise volume, with H<sub>2</sub>O, to 50 mL.
- VII. Store at -20°C

### 11. Grp12- Important minerals group: MgSO<sub>4</sub>·7H<sub>2</sub>O (50 g/L), FeSO<sub>4</sub>·7H<sub>2</sub>O (0.2 g/L), ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.25 g/L)

- I. Add 2.5g of MgSO<sub>4</sub> to flask with stir-bar
- II. Add 30 mL of H<sub>2</sub>O to flask; CS
- III. Add 10mg of FeSO<sub>4</sub>; CS
- IV. Add 12.5mg of ZnSO<sub>4</sub>; CS until dissolved.
- V. Raise volume, with H<sub>2</sub>O, to 50 mL.
- VI. Store at -20°C

### 12. Grp13- Other Vitamin group: Folic acid (0.075 g/L), p-Aminobenzoic acid (0.0075 g/L)

- I. Make (0.75 g/L) solution of Folic acid by initially adding 37.5mg of Folic acid and 30 mL of H<sub>2</sub>O into a separate 125 mL flask. Add 200 uL of 1N NaOH; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- II. Make (0.75 g/L) solution of p-Aminobenzoic acid by initially adding 37.5mg of p-Aminobenzoic acid and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- III. Add 5 mL of (0.75 g/L) Folic acid solution and 500 uL of (0.75 g/L) p-Aminobenzoic acid solution to a new 125 mL flask.
- IV. Raise volume, with H<sub>2</sub>O, to 50 mL and Stir, by hand.
- V. Store at -20°C

### 13. Grp14- Fatty acid group: Potassium acetate (90.1 g/L), Lipoic acid aka Thioctic acid (0.1 g/L), Tween 80 (50 g/L)

- I. Make (1.0 g/L) solution of Thioctic acid by initially adding 50mg of Thioctic acid and 30 mL of H<sub>2</sub>O into a separate 125 mL flask. Add 500 uL of 1N NaOH; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- II. Add 2.5g of Tween 80 to flask with stir-bar
- III. Add 30 mL of H<sub>2</sub>O to flask
- IV. Stir solution over the heating-plate at low heat until dissolved. Quickly removed from heat once it dissolved.
- V. Cool solution for ~5mins
- VI. Add 4.5g of Potassium acetate; CS
- VII. Add 5 mL of (1.0 g/L) Thioctic acid solution to a new 125 mL flask
- VIII. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IX. Store at -20°C

### 14. Grp15- Nucleic acid base group: Adenine (1.5 g/L), Guanine (0.75 g/L), Uracil (3 g/L), Xanthine (0.5 g/L)

- I. Add 75mg of Adenine to flask with stir-bar
- II. Add 20 mL of H<sub>2</sub>O to flask; CS.
- III. Add 600 uL of 1N NaOH, drop by drop; CS until precipitate was dissolved.
- IV. Add 37.5mg of Guanine; CS
- V. Add 1.9 mL of 1N NaOH, drop by drop; CS until precipitate was dissolved.
- VI. Add 150mg of Uracil; CS
- VII. Add 25mg of Xanthine; CS
- VIII. Add 500 uL of 1N NaOH, drop by drop, until precipitate was dissolved.
- IX. Raise volume, with H<sub>2</sub>O, to 50 mL.
- X. Store at -20°C

## Zhang-Mills-Block 1 (ZMB1) Media

### 15. Grp16- Other buffer group: MOPS (500 g/L), Tricine (50 g/L)

- I. Add 25g of MOPS to flask with stir-bar
- II. Add 15 mL of H<sub>2</sub>O to flask; CS
- III. Add 2.5g of Tricine; CS until dissolved.
- IV. Raise volume, with H<sub>2</sub>O, to 50 mL.
- V. Store at -20°C

### 16. Grp17- Traced mineral group: (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O (0.025 g/L), MnSO<sub>4</sub>·4H<sub>2</sub>O (0.05 g/L), CaCl<sub>2</sub>·2H<sub>2</sub>O (5 g/L), CoSO<sub>4</sub>·6H<sub>2</sub>O (0.03 g/L), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.025 g/L), H<sub>3</sub>BO<sub>3</sub> (0.1 g/L), K<sub>2</sub>SO<sub>4</sub> (3 g/L), KI (0.015 g/L)

- I. Make (0.25 g/L) solution of (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O by initially adding 12.5mg (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- II. Make (0.5 g/L) solution of MnSO<sub>4</sub>·4H<sub>2</sub>O by initially adding 25mg of MnSO<sub>4</sub>·4H<sub>2</sub>O and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- III. Make (0.3 g/L) solution of CoSO<sub>4</sub>·6H<sub>2</sub>O by initially adding 15mg of CoSO<sub>4</sub>·6H<sub>2</sub>O and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IV. Make (0.25 g/L) solution of CuSO<sub>4</sub>·5H<sub>2</sub>O by initially adding 15mg of CuSO<sub>4</sub>·5H<sub>2</sub>O and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- V. Make (1.0 g/L) solution of H<sub>3</sub>BO<sub>3</sub> by initially adding 50mg of H<sub>3</sub>BO<sub>3</sub> and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- VI. Make (1.5 g/L) solution of KI by initially adding 75mg of KI and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL; CS
- VII. In a new 125 mL flask with stir-bar, Add 250mg of CaCl<sub>2</sub>·2H<sub>2</sub>O.
- VIII. Add 5 mL of (0.25 g/L) (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O solution, 5 mL of (0.5 g/L) MnSO<sub>4</sub>·4H<sub>2</sub>O solution, 5 mL of (0.3 g/L) CoSO<sub>4</sub>·6H<sub>2</sub>O solution, 5 mL of (0.25 g/L) CuSO<sub>4</sub>·5H<sub>2</sub>O solution, 5 mL of (1.0 g/L) H<sub>3</sub>BO<sub>3</sub> solution, and 500 uL of (0.15g/L) KI solution
- IX. Stir solution until dissolved
- X. Add 150mg of K<sub>2</sub>SO<sub>4</sub>; CS
- XI. Raise volume, with H<sub>2</sub>O, to 50 mL.
- XII. Store at -20°C

### 17. Grp18- Chelator group: EDTA (1.0 g/L), NTA aka Nitrilotriacetic acid (1.0 g/L)

- I. Add 50mg of EDTA into flask with beaker
- II. Add 10 mL of H<sub>2</sub>O to flask; CS
- III. Add 50mg of NTA; CS
- IV. Add 500 uL of 1N NaOH; CS until dissolved.
- V. Raise volume, with H<sub>2</sub>O, to 50 mL.
- VI. Store at -20°C

### 18. Grp19- Other component group- Glutathione (0.75 g/L), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (50 g/L), NaCl (150 g/L)

- I. Add 37.5mg of Glutathione into flask with beaker
- II. Add 30 mL of H<sub>2</sub>O to flask; CS
- III. Add 2.5g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; CS
- VII. Add 7.5g of NaCl; CS until dissolved.
- IV. Raise volume, with H<sub>2</sub>O, to 50 mL.
- V. Store at -20°C

### 19. Grp20- EAA#7: L-Tyrosine (7.5 g/L)

- I. Add 375mg of L-Tyrosine to flask with stir-bar
- II. Add 4 mL of 1N NaOH and 4 mL of H<sub>2</sub>O; Stir until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IV. Store at -20°C

### 20. Grp21- EAA#8: Biotin (0.5 g/L)

- I. Add 25mg of Biotin to flask with stir-bar
- II. Add 500 uL of 1N NaOH and 4 mL of H<sub>2</sub>O; Stir until dissolved.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL; CS
- IV. Store at -20°C

### 21. Grp22- EAA#9: Thiamine HCl (0.075 g/L)

- I. Make (0.75 g/L) solution of Thiamine HCl by initially adding 37.5mg of Thiamine HCl and 30 mL of H<sub>2</sub>O into a separate 125 mL flask; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- II. In a new 125 mL flask, Add 5 mL of (0.75 g/L) Thiamine HCl solution.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL.
- IV. Store at -20°C

### 22. Grp23- EAA#10: Riboflavin (0.075 g/L)

- I. Make (0.75 g/L) solution of Riboflavin by initially adding 37.5mg of Riboflavin and 30 mL of H<sub>2</sub>O into a separate 125 mL flask. Add 600 uL of 1N NaOH; CS until dissolved. Raise volume, with H<sub>2</sub>O, to 50 mL.
- II. In a new 125 mL flask, Add 5 mL of (0.75 g/L) Riboflavin solution.
- III. Raise volume, with H<sub>2</sub>O, to 50 mL.

## Zhang-Mills-Block 1 (ZMB1) Media

- IV. Store at -20°C

### 100 mL of 1N NaOH

- I. Add 70 mL of H<sub>2</sub>O to beaker. Placed beaker in bucket of ice
- II. Add, slowly, 4g of NaOH
- III. Stir until dissolved
- IV. Poured solution into glass graduated cylinder
- V. Raise final volume to 100 mL with H<sub>2</sub>O
- VI. Poured final solution into new 500 mL bottle.
- VII. Store at room temp

### 100 mL of 10N NaOH

- I. Add 70 mL of H<sub>2</sub>O to beaker. Placed beaker in bucket of ice
- II. Add, slowly, 40g of NaOH
- III. Stir until dissolved
- IV. Poured solution into glass graduated cylinder
- V. Raise final volume to 100 mL with H<sub>2</sub>O
- VI. Poured final solution into new 500 mL bottle
- VII. Store at room temp

### To make 50 mL of ZMB1 media:

- I. Add in the following order of **descending group number**:

Group no.	Volume ( mL) Add	Group no.	Volume ( mL) Add	Group no.	Volume ( mL) Add
2	1.7	11	0.6	20	2
3	0.6	12	1	21	0.6
4	2	13	0.373	22	0.373
5	0.2	14	0.5	23	0.6
6	2	15	0.367		
7	1.8	16	1.5		
8	1	17	0.38		
9	3	18	0.375		
10	2	19	1		

- II. Volume of media was 25.6 mL after adding groups #2 thru #23. Next, add 14.4 mL of H<sub>2</sub>O and stir using magnetic stir-bar. Resulted in volume of 40 mL with pH of 6.76.
- III. While stirring, add 100 uL of 10N NaOH at a time, drop by drop, to adjust the final pH to 7.8. Amount of 10N NaOH used may vary.
- IV. Add H<sub>2</sub>O to raise final volume to 50 mL.  
- final volume of H<sub>2</sub>O used may vary depending on the amount of NaOH used to adjust pH.
- V. SFS into a sterile 50 mL falcon tube.
- VI. Covered in aluminum foil.
- VII. Store at 4°C