

Growth media for biofilm establishment and glucose challenge

Unbuffered CDM medium modified from (McLean, Ona et al. 2008) and more recently (McLean, Fansler et al. 2012) was prepared for the glucose challenge with the following media components: 0.8 g $(\text{NH}_4)_2\text{SO}_4$, 0.6 g NaCl, 0.16 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.01 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 5.0 g KH_2PO_4 , 0.3 g L-Cysteine hydrochloride, 20 mL vitamin mix and 1 mL mineral mix. Vitamin mix was prepared from 100X stock solution consisting of: 0.002 g biotin (d-biotin), 0.002 g folic acid, 0.001 g pyridoxine hydrochloride, 0.005 g riboflavin, 0.005 g thiamine hydrochloride (1.0 H_2O), 0.005 g nicotinic acid, 0.005 g d-pantothenic acid, hemicalcium salt, 0.005 g d-pantothenic acid, hemicalcium salt, 0.0001 g vitamin B12, 0.005 g p-aminobenzoic acid, 0.005 g thiocetic acid. The mineral mix was prepared from a 100X stock solution (1L) as follows: 1.5 g nitrilotriacetic acid (dissolved in NaOH to pH 8), 3 g magnesium sulfate heptahydrate, 0.5 g manganese sulfate monohydrate, 1 g sodium chloride, 0.1 g ferrous sulfate heptahydrate, 0.1 g calcium chloride dehydrate, 0.1 g cobalt chloride hexahydrate, 0.13 g zinc chloride, 0.01 g cupric sulfate pentahydrate, 0.01 g aluminum potassium disulfate dodecahydrate 0.01 g boric acid, 0.025 g sodium molybdate dehydrate, 0.024 g nickel chloride hexahydrate, 0.025 g sodium tungstate. pH was set to 7 in each stock solution prior to filter sterilizing (pore size: 0.02 μm). Mineral and vitamin mixes were then added to the autoclaved main media components. Buffered CDM medium, used for the starvation period was prepared in a similar manner but with 3.0 g KH_2PO_4 and 2.5 g K_2HPO_4 and the pH adjusted to 7.

Keep stored at 4oC and wrap in tinfoil.

CDM2 MEDIA

| <u>Component</u> | <u>g/l</u> | <u>Molar concentration</u> |
|---------------------------------------|-------------------|-----------------------------------|
| NH4 sulfate | 0.8 | 6.0542 millimoles |
| NaCl | 0.6 | 10.2669 millimoles |
| Ascorbic acid | 0.5 | 2.524 millimoles |
| MgCl ₂ · 6H ₂ O | 0.16 | 786.9756 micromoles |
| CaCl ₂ · 2H ₂ O | 0.01 | 68.0272 micromoles |
| KH ₂ PO ₄ | 3 | 22.0426 millimoles |
| K ₂ HPO ₄ | 2.5 | 14.3513 millimoles |
| Cysteine HCl | 0.3 | 1.7084 millimoles |
| *Vitamin mix | 20ml | |

***Mineral Mix**

1mL

was 6.93 after all additions.

filter sterilize seemed to give best results

| | | |
|--------------------|-----|--------------------|
| Glucose * optional | 2.5 | 13.8889 millimoles |
|--------------------|-----|--------------------|

For low pH or unbuffered media remove
K₂HPO₄ and only add KH₂PO₄

| | | |
|-------------------------------------|------------------|------------------------|
| KH₂PO₄ | 5 g/L | 36.4 millimoles |
|-------------------------------------|------------------|------------------------|

The mineral and vitamin mix are made in the lab
from stock chemicals but are very similar to
wolfes vitamin and wolfes mineral solutions
available at ATCC
<http://www.atcc.org/Products/All/MD-VS.aspx#generalinformation>

<http://www.atcc.org/Products/All/MD-TMS.aspx#generalinformation>

Vitamin Solution

| Chemical Description | FW | g/L 100X stock | Formula | Vendor/Cat # | Final conc. in medium nM |
|---------------------------------------|------|----------------------|---------------------|--------------|-----------------------------------|
| •biotin (d-biotin) | 244 | 0 | C10H16N2O3 S | Sigma B-4639 | 81.87 |
| •folic acid | 441 | 0 | C19H19N7O6 | Sigma F-7876 | 45.34 |
| •pyridoxine HCl | 206 | 0.01 | C8H12ClNO3 | Sigma P-9755 | 486.38 |
| •riboflavin | 376 | 0.01 | C17H20N4O6 | Sigma R-4500 | 132.84 |
| •thiamine HCl 1.0 H2O | 355 | 0.01 | C18H18Cl2N4 OS | Sigma T-4625 | 140.73 |
| •nicotinic acid | 123 | 0.01 | C6H5NO2 | Sigma N-4126 | 406.17 |
| •d-pantothenic acid, hemicalcium salt | 238 | 0.01 | C9H16NO5. 1/2Ca | Sigma P-2250 | 209.82 |
| •B12 | 1355 | 0 | C63H88CoN1 4O14P | Sigma V-2876 | 0.74 |
| •p-aminobenzoic acid | 137 | 0.01 | C7H7NO2 | Sigma A-9878 | 364.62 |
| •thioctic acid | 206 | 0.01 | C8H14O2S2 | Sigma T-5625 | 242.37 |

Set pH to 7.0 after all components have been added using NaOH or HCl.

Mineral Solution

| Chemical Description | FW | g/L 100X stock | Formula | Vendor/Cat # | 10mL/L final conc. in medium µM | 1mL/L final conc. in medium µM |
|---|------|----------------------|--------------------|------------------|---|---|
| •nitrilotriacetic acid(a) (dissolve with NaOH to pH 8) | 191 | 1.5 | C6H9NO3 | Sigma N-9877 | 78.49 | 7.849 |
| •magnesium sulfate heptahydrate | 246 | 3 | MgSO4 7H2O | Aldrich 23,039-1 | 121.71 | 12.171 |
| •manganese sulfate monohydrate | 169 | 0.5 | MnSO4 H2O | Aldrich 22,128-7 | 29.58 | 2.958 |
| •sodium chloride | 58.4 | 1 | NaCl | Sigma S-3014 | 171.12 | 17.112 |
| •ferrous sulfate heptahydrate | 278 | 0.1 | FeSO4 7H2O | Sigma F-8633 | 3.6 | 0.36 |
| •calcium chloride dihydrate | 147 | 0.1 | CaCl2 2H2O | Sigma C-3881 | 6.8 | 0.68 |
| •cobalt chloride hexahydrate | 238 | 0.1 | CoCl2 6H2O | Sigma C-3169 | 4.2 | 0.42 |
| •zinc chloride | 136 | 0.13 | ZnCl2 | Sigma Z-3500 | 9.54 | 0.954 |
| •cupric sulfate pentahydrate | 250 | 0.01 | CuSO4 5H2O | Sigma C-6283 | 0.4 | 0.04 |
| •aluminum potassium disulfate dodecahydrate | 474 | 0.01 | AlK(SO4)2 12H2O | Sigma A-7167 | 0.21 | 0.021 |
| •boric acid | 61.8 | 0.01 | H3BO3 | Sigma B-6768 | 1.62 | 0.162 |
| •sodium molybdate dihydrate | 242 | 0.03 | Na2MoO4 2H2O | Aldrich 22,184-8 | 1.03 | 0.103 |

McLean, J. S., S. J. Fansler, P. D. Majors, K. McAteer, L. Z. Allen, M. E. Shirtliff, R. Lux and W. Shi (2012). "Identifying low pH active and lactate-utilizing taxa within oral microbiome communities from healthy children using stable isotope probing techniques." PLoS One **7**(3): e32219.

McLean, J. S., O. N. Ona and P. D. Majors (2008). "Correlated biofilm imaging, transport and metabolism measurements via combined nuclear magnetic resonance and confocal microscopy." ISME J **2**(2): 121-131.