

### Nitrogen-free liquid medium for tubes

*\*Make sure tubes and stoppers are in the anaerobic chamber at least 1 hour ahead of time to allow oxygen to diffuse from them.\**

	<u>100 ml:</u>	<u>200 ml:</u>	<u>500 ml:</u>	<u>600 ml</u>
<u>Combine before boiling:</u>				
H <sub>2</sub> O	50 ml	100 ml	250 ml	300 ml
N-free General Salts solution	50 ml	100 ml	250 ml	300 ml
NaHCO <sub>3</sub>	0.5 g	1 g	2.5 g	3 g
NaCl	2.2 g	4.4 g	11 g	13.2 g
K <sub>2</sub> HPO <sub>4</sub> soln	1 ml	2 ml	5 ml	6 ml
FeSO <sub>4</sub> soln	0.5 ml	1 ml	2.5 ml	3 ml
Trace Minerals (1,000X)	0.1 ml	0.2 ml	0.5 ml	0.6 ml
Vitamin soln (100X)	1 ml	2 ml	5 ml	6 ml
Resazurin soln	0.1 ml	0.2 ml	0.5 ml	0.6 ml
NaAcetate·3H <sub>2</sub> O	0.14 g	0.28 g	0.7 g	0.84 g
<i>*Add just before boiling:*</i>				
dithiothreitol	0.05 g	0.1 g	0.25 g	0.3 g

- (1) Put stopper in flask and insert gassing canula alongside stopper. Put medium under stream of N<sub>2</sub>CO<sub>2</sub>.
- (2) Bring to boil. Then turn off heat and continue stream of N<sub>2</sub>CO<sub>2</sub> for 5-10 minutes, swirling occasionally, until medium changes from pink to clear, precipitate has gone away, and medium has cooled to the point where you can carry it comfortably by hand.
- (4) Pull out gassing canula and simultaneously push in stopper. Tape down stopper. Turn off N<sub>2</sub>CO<sub>2</sub>. Bring flask into anaerobic chamber.
- (5) Dispense **5 ml** of medium per tube. Make sure to swirl flask periodically to keep any precipitate evenly suspended while dispensing.
- (6) Once you are done dispensing medium, insert rubber stoppers in tubes and take out of anaerobic chamber.

(7) Crimp seal tubes. Gas exchange with 80:20 N<sub>2</sub>:CO<sub>2</sub> as follows:

- (a) Turn on N<sub>2</sub>:CO<sub>2</sub> and flush out gassing lines. Set pressure to 15 psi at the tank regulator.
- (b) Put needles through stoppers. For the gassing station in F324, which does not have valves on each individual gassing line, all ten lines must be inserted into sealed tubes in order to prevent intake of air when evacuating. For the gassing station in F329, any unused gassing lines may simply be turned off with the toggle valve.
- (c) After pressure has equilibrated into tubes (meter stabilizes at approximately 15 psi), make sure there are no leaks by setting 3-way valve to middle position and making sure pressure does not drop over time. Only then may you evacuate tubes.
- (d) Turn on vacuum pump. Vacuum-fill-vacuum-fill-vacuum-fill (three times) by moving three-way valve between vacuum and pressurize positions. Pause ten seconds between each switching of the valve. After final pressurization, remove gassing lines from tubes one at a time, pushing each needle into the rubber “pin-cusion” or switching off each toggle valve (room F329).

(8) Autoclave

- (9) After autoclaving, before medium cools, carefully invert tubes several times. You can do this by using something flat to hold the tubes in the rack and inverting the whole rack. This helps decrease precipitate after medium cools.

Before use, add Na<sub>2</sub>S, release N<sub>2</sub>:CO<sub>2</sub> to atmospheric pressure by inserting a needle through the stopper, and pressurize with H<sub>2</sub>:CO<sub>2</sub>.