

## 1001. BASAL MEDIUM

NaHCO <sub>3</sub>	2.500	g
NH <sub>4</sub> Cl	0.250	g
NaH <sub>2</sub> PO <sub>4</sub> x H <sub>2</sub> O	0.600	g
KCl	0.100	g
Vitamin Mix (see below)	10.000	ml
Mineral Mix (see below)	10.000	ml
Distilled water	980.000	ml

This medium should not be exposed to direct sunlight! Add all basal medium ingredients. Bring the final volume of the medium to 1.0 liter. Dispense to appropriate culture containers. Bubble the medium with 80:20 N<sub>2</sub>:CO<sub>2</sub> (final pH should be 6.8 to 7.0) - approximately 10 ml of media (anaerobic culture tube) should be gassed for 5 min in the aqueous phase (bubbled) and the headspace gassed for one minute prior to sealing the container. Sterilize per usual procedure. Add electron donor (Acetate-final conc. of 10 mM-recipe below) and electron acceptor (Fe(III)NTA-final conc. of 10 mM-recipe below) from sterile, anaerobic stock solutions using a sterile syringe and needle flushed with anaerobic gas. Store out of direct light.

### *1.0 M Acetate Stock Solution:*

Add 13.6 g sodium acetate to ca. 80 ml distilled water. Bring to final volume of 100 ml. Bubble with anaerobic gas (nitrogen) for 45 min. Seal and sterilize.

### *500 mM Fe(III)NTA Stock solution:*

Add 8.2 g NaHCO<sub>3</sub> to ca. 70 ml distilled water. Add 12.8 g of Na<sub>3</sub>Nitriilotriacetic acid (NTA) to ca. 70 ml distilled water. Add 13.5 g FeCl<sub>3</sub> x 6 H<sub>2</sub>O to ca 70 ml distilled water. Adjust pH to 6.5 using 10N NaOH. Bring solution to final volume of 100 ml. Ingredients will go into solution after stirring for ca. 15 min. Bubble with anaerobic gas (N<sub>2</sub>) for 45 min and then filter sterilize (0.2 µm filter) into a sterile, anaerobic, serum bottle.

### *Vitamin mixture:*

Biotin	2.000	mg
Folic acid	2.000	mg
Pyridoxine HCl	10.000	mg
Riboflavin	5.000	mg
Thiamine	5.000	mg
Nicotinic acid	5.000	mg
Pantothenic acid	5.000	mg
Vitamin B <sub>12</sub>	0.100	mg
p-aminobenzoic acid	5.000	mg
Thioctic acid	5.000	mg
Distilled water	1000.000	ml

*Continued on next page*

*Mineral mixture:*

NTA	1.500	g
MgSO <sub>4</sub>	3.000	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.500	g
NaCl	1.000	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.100	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.100	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.100	g
ZnCl <sub>2</sub>	0.130	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.010	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.010	g
H <sub>3</sub> BO <sub>3</sub>	0.010	g
Na <sub>2</sub> MoO <sub>4</sub>	0.025	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.024	g
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.025	g
Distilled water	1000.000	ml