

**1210: GEOGLOBUS MEDIUM**

NaCl	18.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.00	g
KCl	0.33	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.33	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.50	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
<b>Amorphous Fe(OH)<sub>3</sub></b>	200.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Na-acetate	1.50	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
Yeast extract	0.20	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Distilled water	800.00	ml

Dissolve ingredients except carbonate, acetate, hydrogenphosphate, yeast extract, vitamins and ferric iron hydroxide sludge. Suspend pellet of ferric iron hydroxide in medium and sparge with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Thereafter, dispense suspension under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave for 30 min. Add acetate, hydrogenphosphate, yeast extract and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. The pH of the complete medium should be at 6.5 - 6.8.

**Trace element solution SL-10 (from medium 320)**

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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### Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4.00	mg
Distilled water	1000.00	ml

### Amorphous Fe(OH)<sub>3</sub> (from medium 1210)

FeCl <sub>3</sub> x 6 H <sub>2</sub> O (60.00 g/l)	320.00	ml
NaOH (10% w/v), adjust if required	80.00	ml

Slowly titrate 320 ml of a FeCl<sub>3</sub> x 6 H<sub>2</sub>O stock solution (60.00 g/l) with 10% (w/v) NaOH to pH 8.0-8.5 under agitation (use magnetic stirrer). Total amount of added NaOH approx. 80 - 100 ml. The precipitated Fe(OH)<sub>3</sub> should be stored at room temperature overnight with surface covered with water. Thereafter, centrifuge at 2000 rpm for 5 min and discard the supernatant. Wash several times with distilled water. Resuspend the pellet in medium as described above. For storage autoclave under 100% N<sub>2</sub> atmosphere.

### Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml