

## 946: NAUTILIA MEDIUM

<b>Synthetic seawater (2 x conc.)</b>	500.00	ml
NH <sub>4</sub> Cl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Na-formate	3.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.60	g
Distilled water	500.00	ml

Dissolve ingredients (except sulfur, carbonate, formate, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur. For sterilization heat cultivation vessels for 1 - 2 hours to 90 - 100°C in a water bath on each of three successive days. Prior to inoculation add carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and formate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary.

### Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

### Synthetic seawater (2 x conc.) (from medium 795)

NaCl	55.40	g
SrCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	14.00	g
KI	0.10	mg
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	11.00	g
Na <sub>3</sub> -citrate	20.00	mg
KCl	1.30	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.50	g
NaBr	0.20	g
H <sub>3</sub> BO <sub>3</sub>	0.06	g
Distilled water	1000.00	ml

### 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