

**792c. NAUTILIA NITRATIREDUCTENS MEDIUM****Solution A:**

NaCl	20.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	2.75	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.75	g
KCl	0.33	g
NaBr	0.05	g
H <sub>3</sub> BO <sub>3</sub>	15.00	mg
SrCl <sub>2</sub> x 6 H <sub>2</sub> O solution (0.1% w/v)	7.00	ml
KI solution (0.01% w/v)	0.50	ml
Wolfe's mineral elixir (see medium 792)	1.00	ml
Distilled water	950.00	ml

**Solution B:**

KNO <sub>3</sub>	1.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
Distilled water	10.00	ml

**Solution C:**

Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Distilled water	20.00	ml

**Solution D:**

Wolin's vitamin solution (see medium 141)	10.00	ml
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**Solution E:**

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.45	g
Distilled water	15.00	ml

Sparge *solution A* with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic serum vials (e.g., 20 ml medium in 100 ml bottles) and autoclave. *Solutions B* and *C* are autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. *Solution D* is prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. *Solution E* is autoclaved under 100% N<sub>2</sub> gas atmosphere. To complete the medium appropriate amounts of *solutions B* to *E* are added to *solution A*. Adjust pH of the complete medium to 6.5. After inoculation pressurize vials to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

*Note: Solution A has to be prepared freshly. It cannot be stored for a long period of time.*