

**961. THERMOVIBRIO MEDIUM**

Na-resazurin solution (0.1% w/v)	0.50	ml
KNO <sub>3</sub>	1.00	g
Yeast extract (OXOID)	1.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Synthetic seawater (see below)	1000.00	ml

Dissolve resazurin in seawater, then sparge medium with 80% H<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 serum vials (e.g., 20 ml medium per 100 ml bottle) and autoclave. Add nitrate, yeast extract and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 6.5, if necessary. After inoculation pressurize bottles to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Note: *Alternatively, aged filtered seawater can be used instead of synthetic seawater.*

*Synthetic seawater:*

NaCl	27.70	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	7.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	5.50	g
KCl	0.65	g
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.25	g
H <sub>3</sub> BO <sub>3</sub>	0.03	g
NaBr	0.10	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.75	g
KI	0.05	mg
SrCl <sub>2</sub> x 6 H <sub>2</sub> O	15.00	mg
Distilled water	1000.00	ml