

**1210c. THERMOSULFURIPHILUS 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
KNO <sub>3</sub>	1.00	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Sulfur, powder	5.00	g
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
Vitamin solution (see medium 141)	1.00	ml
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	3.50	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur, carbonate, hydrogenphosphate, vitamins and thiosulfate, 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 containing already the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Add hydrogenphosphate, vitamins and thiosulfate 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. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. The pH of the complete medium should be at 6.5 – 6.8.