

Main sol. 827

NH ₄ Cl	0.33	g
KCl	0.33	g
KH ₂ PO ₄	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g

Trace element solution SL-10	1.00	ml
-------------------------------------	------	----

Sodium resazurin (0.1% w/v)	0.50	ml
-----------------------------	------	----

Sulfur (powdered)	10.00	g
-------------------	-------	---

Na ₂ CO ₃	1.00	g
---------------------------------	------	---

Yeast extract	0.50	g
---------------	------	---

D-Glucose	5.00	g
-----------	------	---

Wolin's vitamin solution (10x)	1.00	ml
---------------------------------------	------	----

Na ₂ S x 9 H ₂ O	0.50	g
--	------	---

Distilled water	1000.00	ml
-----------------	---------	----

Dissolve ingredients except sulfur, carbonate, yeast extract, glucose, vitamins, and sulfide, then sparge medium with 80% N₂ and 20% CO₂ 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. Heat the medium vials to 90-100°C for 1 hour on each of 3 successive days to sterilize the medium. Add yeast extract, glucose, vitamins (sterilized by filtration), and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.