

Main sol. 480c

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

Trace element solution SL-10

MOPS buffer (SIGMA M3183)	1.00	ml
Yeast extract	3.90	g
Sodium resazurin (0.1% w/v)	0.10	g
Sulfur (powder)	0.50	ml
Na-DL-lactate	10.00	g
Na ₂ CO ₃	2.50	g
Na ₂ CO ₃	1.00	g

Wolin's vitamin solution (10x)

Na ₂ S x 9 H ₂ O	1.00	ml
Distilled water	0.50	g
	1000.00	ml

Dissolve ingredients (except sulfur, lactate, carbonate, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 5.9 and sterilize medium by heating for 2- 3 hours in a boiling water bath on each of 3 successive days. Add lactate, 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 adjust pH of complete medium to 6.8 - 7.0, if necessary.