

1304: SULFURITALEA MEDIUM

MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.10	g
NaNO ₃	1.70	g
(NH ₄) ₂ SO ₄	0.10	g
KH ₂ PO ₄	0.10	g
KCl	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na ₂ S ₂ O ₃ x 5 H ₂ O	0.40	g
NaHCO ₃	5.00	g
Wolin's vitamin solution	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients except trace elements, thiosulfate, bicarbonate and vitamins, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to remove dissolved oxygen and to saturate the solution with CO₂. Dispense solution under 80% N₂ and 20% CO₂ gas atmosphere in vials suitable for anaerobic cultures (e.g., Balch-type tubes) to 30% of volume. Close vials with butyl rubber septa and autoclave. Add trace element solution, selenite-tungstate solution and vitamins from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 6.7 - 7.0, if necessary.
2. After inoculation, add 1 bar overpressure of sterile 100% H₂ gas.
3. Note: Use at least 10% (v/v) as inoculum.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na ₂ SeO ₃ x 5 H ₂ O	3.00	mg
Na ₂ WO ₄ x 2 H ₂ O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B ₁₂	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml