

1599: CONTUBERNALIS MEDIUM

NH ₄ Cl	0.50	g
KH ₂ PO ₄	0.20	g
MgCl ₂ x 6 H ₂ O	0.10	g
KCl	0.20	g
NaCl	4.20	g
Na ₂ SO ₄	3.00	g
Yeast extract	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	3.30	g
NaCO ₃	6.50	g
Na-acetate	3.00	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonates, acetate and sulfide) and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Then, add and dissolve bicarbonate and carbonate, adjust pH to 9.5, dispense under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add acetate and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Adjust pH of complete medium to 9.5 - 9.7, if necessary.

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.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
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$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$	3.00	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$	4.00	mg
Distilled water	1000.00	ml