

1030. SMITHELLA MEDIUM

NH ₄ Cl	1.0	g
MgCl ₂ x 6 H ₂ O	1.0	g
CaCl ₂ x 2 H ₂ O	0.4	g
KH ₂ PO ₄	0.4	g
Wolfe's mineral elixier (see medium 792)	1.0	ml
Yeast extract	2.0	g
Trypticase peptone (BD BBL)	2.0	g
Na-resazurin solution (0.1% w/v)	0.5	ml
NaHCO ₃	2.0	g
Na-crotonate solution (1 M, see medium 870)	20.0	ml
Vitamin solution (see medium 141)	10.0	ml
2-Mercaptoethanesulfonic acid (coenzyme M)	0.5	g
Na ₂ S x 9 H ₂ O	0.3	g
Distilled water	1000.0	ml

Dissolve ingredients (except bicarbonate, crotonate, coenzyme M, 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 Hungate-type tubes or serum vials (e.g., 5 ml medium in Balch-type tubes) and autoclave. Add crotonate, coenzyme M, vitamins and sulfide 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. Stock solutions of crotonate, coenzyme M and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2.

Note: Use 10% (v/v) as inoculum.