1030. SMITHELLA MEDIUM

NH₄Cl 1.0 g
MgCl₂ x 6 H₂O 1.0 g
CaCl₂ x 2 H₂O 0.4 g
KH₂PO₄ 0.4 g
Wolfe's mineral elixier (see medium 792) 1.0 ml
Yeast extract 2.0 g
Tryptase peptone (BD BBL) 2.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
Na₂CO₃ 1.0 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
Na₂S x 9 H₂O 0.3 g
Distilled water 1000.0 ml

Dissolve ingredients (except carbonate, 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 carbonate 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.