

**1030. SMITHELLA MEDIUM**

NH <sub>4</sub> Cl	1.0	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	1.0	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.4	g
KH <sub>2</sub> PO <sub>4</sub>	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
Na <sub>2</sub> CO <sub>3</sub>	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
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.3	g
Distilled water	1000.0	ml

Dissolve ingredients (except carbonate, crotonate, coenzyme M, vitamins and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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.*