335. EUBACTERIUM OXIDOREDUCTENS MEDIUM

Solution A:
Mineral solution (see below) 50.00 ml
Trace element solution SL-10 (see medium 320) 1.00 ml
Yeast extract 2.00 g
Crotonic acid 5.00 g
Vitamin solution (see medium 503) 1.00 ml
Resazurin 1.00 mg
Distilled water 840.00 ml

Adjust pH to 6.9 before autoclaving.

Solution B:
NaHCO₃ 5.00 g
Distilled water 100.00 ml

Solution C:
L-Cysteine-HCl x H₂O 0.24 g
Distilled water 10.00 ml

Solution D:
Na₂S x 9 H₂O 78.00 mg
Distilled water 1.00 ml

Prepare solution A under 80% N₂ and 20% CO₂ gas mixture, distribute 8.9 ml per Hungate-type tube under same gas atmosphere and autoclave. Filter sterilize NaHCO₃ solution and gas for 20 min with 80% N₂ and 20% CO₂ gas mixture. Autoclave cysteine and sulfide under 100% N₂ gas. Complete medium by adding the solutions B, C, and D with a syringe to solution A. Adjust final pH of medium to 7.0 - 7.5.

Mineral solution:
KH₂PO₄ 10.0 g
MgCl₂ x 6 H₂O 6.6 g
NaCl 8.0 g
NH₄Cl 8.0 g
CaCl₂ x 2 H₂O 1.0 g
Distilled water 1000.0 ml