1665. DESULFAMPLUS MEDIUM

NaCl 20.0 g
MgCl₂ x 6 H₂O 6.0 g
Na₂SO₄ 3.0 g
CaCl₂ x 2 H₂O 0.2 g
KCl 0.5 g
NH₄Cl 0.3 g
Wolfe’s mineral elixir (see medium 792) 2.0 ml
HEPES (SIGMA) 2.4 g
Na₂-fumarate 1.0 g
Yeast extract (OXOID) 0.2 g
Na-resazurin solution (0.1% w/v) 0.5 ml
0.5 M K-phosphate buffer, pH 7.0 2.0 ml
NaHCO₃ 1.0 g
FeCl₂ x 4 H₂O solution (0.2% w/v in 0.02 N HCl) 10.0 ml
Vitamin solution (see medium 141) 10.0 ml
L-Cysteine-HCl x H₂O 0.5 g
Distilled water 1000.0 ml

Dissolve ingredients (except phosphate buffer, bicarbonate, ferrous chloride, vitamins and cysteine), adjust pH to 7.2 with NaOH and sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add phosphate buffer, ferrous chloride, vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.2 – 7.5, if necessary.