521. DESULFOMONILE MEDIUM

NH₄Cl 1.0 g
Na₂SO₄ 2.0 g
Na₂S₂O₃ x 5 H₂O 1.0 g
MgSO₄ x 7 H₂O 1.0 g
CaCl₂ x 2 H₂O 0.1 g
KH₂PO₄ 0.5 g
Trace element solution SL-10 (see medium 320) 1.0 ml
Selenite-tungstate solution (see medium 385) 1.0 ml
Yeast extract 1.0 g
Na-resazurin solution (0.1% w/v) 0.5 ml
NaHCO₃ 1.0 g
Vitamin solution (see below) 10.0 ml
Na-pyruvate 2.5 g
Na₂S x 9 H₂O 0.1 g
Distilled water 1000.0 ml

Dissolve ingredients (except bicarbonate, vitamins, pyruvate and sulfide), then sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete the medium by adding vitamins, pyruvate and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Stock solutions of vitamins and pyruvate are sterilized by filtration. Adjust pH of the complete medium to 6.8 - 7.0.

Note: Prior to inoculation 10-20 mg/l sodium dithionite (added from a 5% w/v solution freshly prepared under N₂ and filter-sterilized) can be added to the medium to stimulate growth at the beginning.

Vitamin solution:
Pyridoxine-HCl 5.0 mg
Riboflavin 5.0 mg
Ca-pantothenate 5.0 mg
p-Aminobenzoate 5.0 mg
Thioctic acid 5.0 mg
Nicotinamide 50.0 mg
Biotin 5.0 mg
Folic acid 5.0 mg
Vitamin B₁₂ 5.0 mg
Haemin (dissolved in 0.1 N NaOH) 5.0 mg
1,4-Naphthoquinone (dissolved in 0.1 N NaOH) 20.0 mg
Distilled water 1000.0 ml