1243: PARADESULFITOBACTERIUM MEDIUM

Ferric citrate monohydrate 13.70 g
NaCl 1.00 g
MgCl₂ x 6 H₂O 0.40 g
KH₂PO₄ 0.20 g
NH₄Cl 0.25 g
KCl 0.50 g
CaCl₂ x 2 H₂O 0.15 g

**Modified Wolin's mineral solution** 10.00 ml
NaHCO₃ 2.50 g
Na-pyruvate 2.50 g

**Wolin's vitamin solution (10x)** 1.00 ml
Na₂S x 9 H₂O 0.10 g

Distilled water 1000.00 ml

First dissolve ferric citrate by heating the water. After cooling to room temperature adjust
the pH to 6.0 and dissolve the other ingredients (except bicarbonate, vitamins, pyruvate
and sulfide). Sparge medium with 80% N₂ and 20% CO₂ gas mixture 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. Before inoculation add vitamins,
pyruvate and sulfide from sterile anoxic stock solutions prepared under 100%
N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂
gas mixture. Stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust
pH of complete medium to 6.8 - 7.2, if necessary.

**Modified Wolin's mineral solution** (from medium 141)
Nitrilotriacetic acid 1.50 g
MgSO₄ x 7 H₂O 3.00 g
MnSO₄ x H₂O 0.50 g
NaCl 1.00 g
FeSO₄ x 7 H₂O 0.10 g
CoSO₄ x 7 H₂O 0.18 g
CaCl₂ x 2 H₂O 0.10 g
ZnSO₄ x 7 H₂O 0.18 g
CuSO₄ x 5 H₂O 0.01 g
Al₂(SO₄)₃ x 12 H₂O 0.02 g
H₃BO₃ 0.01 g
Na₂MoO₄ x 2 H₂O 0.01 g
NiCl₂ x 6 H₂O 0.03 g
Na₂SeO₃ x 5 H₂O 0.30 mg
Na₂WO₄ x 2 H₂O 0.40 mg
Distilled water 1000.00 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

**Wolin's vitamin solution (10x)** (from medium 120)
- Biotin: 20.00 mg
- Folic acid: 20.00 mg
- Pyridoxine hydrochloride: 100.00 mg
- Thiamine HCl: 50.00 mg
- Riboflavin: 50.00 mg
- Nicotinic acid: 50.00 mg
- Calcium D-(+)-pantothenate: 50.00 mg
- Vitamin B₁₂: 1.00 mg
- p-Aminobenzoic acid: 50.00 mg
- (DL)-alpha-Lipoic acid: 50.00 mg
- Distilled water: 1000.00 ml