1263. SPIROCHAETA DISSIPATITROPHA MEDIUM

**Ingredients**: 

- **NaCl**: 30.00 g
- **KCl**: 0.20 g
- **K₂HPO₄**: 0.20 g
- **MgCl₂ x 6 H₂O**: 0.10 g
- **NH₄Cl**: 1.00 g
- **Trace element solution (see medium 141)**: 10.00 ml
- **Na-resazurin solution (0.1% w/v)**: 0.50 ml
- **Na₂CO₃**: 2.76 g
- **NaHCO₃**: 24.00 g
- **Yeast extract**: 0.50 g
- **Cellobiose**: 5.00 g
- **Vitamin Solution (see medium 141)**: 2.00 ml
- **Na₂S x 9 H₂O**: 0.40 g
- **Distilled water**: 1000.00 ml

Dissolve ingredients (except carbonate, bicarbonate, yeast extract, cellobiose, vitamins and sulfide), then sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Add solid carbonate and bicarbonate to the medium, dissolve and adjust to pH 9.4. Dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum bottles and autoclave. To the autoclaved medium add yeast extract, cellobiose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere. Stock solutions of vitamins and cellobiose should be sterilized by filtration.