

**1263. SPIROCHAETA DISSIPATITROPHA MEDIUM**

NaCl	30.00	g
KCl	0.20	g
K <sub>2</sub> HPO <sub>4</sub>	0.20	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
NH <sub>4</sub> Cl	1.00	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	2.76	g
NaHCO <sub>3</sub>	24.00	g
Yeast extract	0.50	g
Cellobiose	5.00	g
Vitamin Solution (see medium 141)	2.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, bicarbonate, yeast extract, cellobiose, vitamins and sulfide), then sparge medium with 100% N<sub>2</sub> 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% N<sub>2</sub> 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% N<sub>2</sub> gas atmosphere. Stock solutions of vitamins and cellobiose should be sterilized by filtration.