

700. ALKALIPHILIC SPIROCHAETE MEDIUM

Na ₂ CO ₃	10.0	g
NaHCO ₃	15.0	g
NaCl	10.0	g
K ₂ HPO ₄	0.2	g
NH ₄ Cl	1.0	g
KCl	0.2	g
Na ₂ S x 9 H ₂ O	1.0	g
Yeast extract	0.5	g
Sucrose	5.0	g
Vitamin solution (see medium 141)	10.0	ml
Trace element solution (see medium 141)	1.0	ml
Distilled water	1000.0	ml

Prepare the medium under N₂, omitting Na₂CO₃, Na HCO₃, Na₂S, sucrose and vitamin solution.

Boil the medium and cool under nitrogen, add the Na₂CO₃ and NaHCO₃ and adjust the pH to 9.7 with 6N NaOH (about 15 ml). Sterilize by autoclaving and add the Na₂S (neutralized), vitamins and sucrose from sterile stock solutions.