

1165. SPIROCHAETA AMERICANA MEDIUM

NaCl	30.00	g
Na ₂ CO ₃	2.76	g
NaHCO ₃	24.00	g
KCl	0.20	g
K ₂ HPO ₄	0.20	g
MgCl ₂ x 6 H ₂ O	0.10	g
NH ₄ Cl	1.00	g
Na ₂ S x 9 H ₂ O	0.40	g
Yeast extract	0.50	g
D-glucose	5.00	g
Vitamin Solution (see below)	2.00	ml
Trace element solution (see below)	1.00	ml
Resazurin	1.00	mg
Distilled water	1000.00	ml

Adjust to pH 9.4

Vitamin Solution:

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H ₂ O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B ₁₂	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Trace element solution:

MnCl ₂ x 4 H ₂ O	720.00	mg
Fe(NH ₄)(SO ₄) ₂ x 12 H ₂ O	400.00	mg
FeSO ₄ x 7 H ₂ O	200.00	mg
CoCl ₂ x 6 H ₂ O	200.00	mg
ZnSO ₄ x 7 H ₂ O	200.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	20.00	mg
NiCl ₂	100.00	mg
CuSO ₄ x 5 H ₂ O	20.00	mg

$\text{AlK}(\text{SO}_4)_2 \times 12 \text{H}_2\text{O}$	20.00	mg
H_3BO_3	20.00	mg
HCl	5.00	ml
Distilled water	200.00	ml

Prepare the medium without the Na_2S , yeast extract, glucose, Na_2CO_3 , NaHCO_3 and vitamins. Heat the medium in a boiling water bath and cool under a stream of nitrogen. Add the Na_2CO_3 , and NaHCO_3 to the cooled medium and check the pH. Dispense into appropriate vessels (Hungate tubes or serum bottles) and autoclave. To the sterile medium add Na_2S , yeast extract, and glucose from autoclaved stock solutions. The vitamin solution is filter sterilised and added to the sterilised medium.