

Main sol. 975

NH ₄ Cl	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
KCl	0.33	g
KNO ₃	1.00	g
NaCl	30.00	g
HEPES (10mM)	2.38	g
Yeast extract	0.20	g
Tryptone	1.00	g
Sucrose	2.00	g
Wolin's vitamin solution	1.00	ml
Modified Wolin's mineral solution	1.00	ml
Distilled water	1000.00	ml

Prepare the medium anaerobically, under nitrogen, omitting the CaCl₂, MgCl₂, KNO₃, tryptone, yeast extract, vitamins and sucrose. The pH should be 7.0-7.5. Dispense the medium into vessels suitable for anaerobic growth (Hungate tubes or serum bottles) under an atmosphere of nitrogen and autoclave. To the sterile, cooled medium add, from sterile stock solutions the CaCl₂, MgCl₂, KNO₃, tryptone, yeast extract, vitamins and sucrose. The CaCl₂, MgCl₂, KNO₃, tryptone, yeast extract, and sucrose stock solutions should be autoclaved, while the vitamin solution is sterile filtered. This strain grows anaerobically in the presence of nitrate, but microaerophilic growth has been reported provided the oxygen concentration does not exceed 6 % (v/v). Under anaerobic conditions a redox agent, such as sulphide is not required.