

Main sol. 1344

Taps	17.40	g
Yeast extract	0.10	g
Sodium acetate	1.00	g
Disodium succinate	1.00	g
$\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$	0.10	g
$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$	75.00	mg
$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$	0.20	g
NH_4Cl	0.50	g
KH_2PO_4	0.50	g
$\text{Na}_2\text{-EDTA}$	5.00	mg
Trace elements (A)	1.00	ml
Neutralized sulfide solution (B)	30.00	ml
Bicarbonate solution (C)	30.00	ml
Vitamin solution (D)	1.00	ml
Vitamin B ₁₂ (0.01%)	0.40	ml
Distilled water	1000.00	ml

1. pH 8.3

2. Mix ingredients vitamin B₁₂, vitamin mixture, bicarbonate solution and neutralized sulfide solution. Dispense the medium into suitable culture vessels under a stream of nitrogen gas, seal with butyl rubber stoppers and autoclave at 121°C for 15 min.

Separately autoclave neutralized sulfide solution under a N₂ atmosphere. Sterilize Vitamin B₁₂ solution, vitamin mixture, bicarbonate solution by filtration. Prior to inoculation, aseptically and anaerobically add vitamin B₁₂ solution, vitamin mixture, bicarbonate solution and neutralized sulfide solution.