

Main sol. 1042

Sea Salt (SIGMA)	30.00	g
NaNO ₃	1.00	g
Wolfe's mineral elixir	2.00	ml
VOSO ₄ x 5 H ₂ O (0.01% w/v)	0.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
FeSO ₄ x 7 H ₂ O	0.10	g
NaHCO ₃	1.00	g
Yeast extract	0.50	g
Na-pyruvate	1.00	g
Wolin's vitamin solution (10x)	2.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins), adjust pH to 7.0 and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense the medium under same gas atmosphere into Hungate-type tubes or serum vials and autoclave. Add ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Prepare the stock solution of ferrous sulfate by dissolving 1% (w/v) FeSO₄ x 7 H₂O in 0.1 N H₂SO₄. The stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 6.8-7.0.

2. Note: It may be necessary to add 10-20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under 100% N₂ and filter-sterilized), if the medium is not completely reduced after inoculation.