

705. BACILLUS INFERNUS MEDIUM

NaCl	5.80	g
NH ₄ Cl	1.00	g
K ₂ HPO ₄ x 3 H ₂ O	0.40	g
MgCl ₂ x 6 H ₂ O	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Trace element solution (see below)	10.00	ml
Fe(III)-citrate x 5 H ₂ O	6.70	g
2 N NaOH	20.00	ml
Na-acetate	0.80	g
Na-lactate	1.20	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except ferric citrate, sodium hydroxide, acetate, lactate and coenzyme M. Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Adjust pH to 7.5 with NaOH, dispense under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add ferric citrate (prepare a 10% w/v stock solution by heating in distilled water), sodium hydroxide, acetate, lactate and coenzyme M from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to inoculation the pH of the complete medium should be checked and adjusted to 7.5, if necessary.

Trace element solution:

Na ₂ -EDTA x 2 H ₂ O	0.50	g
CoCl ₂ x 6 H ₂ O	0.15	g
MnCl ₂ x 4 H ₂ O	0.10	g
FeSO ₄ x 7 H ₂ O	0.10	g
ZnCl ₂	0.10	g
AlCl ₃ x 6 H ₂ O	40.00	mg
Na ₂ WO ₄ x 2 H ₂ O	40.00	mg
Na ₂ SeO ₃ x 5 H ₂ O	30.00	mg
NiSO ₄ x 6 H ₂ O	20.00	mg
CuCl ₂ x 2 H ₂ O	20.00	mg
H ₃ BO ₃	10.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	10.00	mg
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7 using 2 N NaOH, then dissolve remaining compounds.