

705: BACILLUS INFERNUS MEDIUM

Ferric citrate monohydrate	5.30	g
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	10.00	ml
Na-acetate	0.80	g
Na-lactate	1.20	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.50	g
Distilled water	1000.00	ml

1. First, dissolve ferric citrate in water by heating and adjust to pH 7.5 with NaOH, then add other medium ingredients except acetate, lactate, and coenzyme M. Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate, lactate, and coenzyme M from sterile anoxic stock solutions prepared under 100% N₂ gas.
2. Prior to inoculation the pH of the complete medium should be checked and adjusted to 7.5, if necessary.

Trace element solution (from medium 705)

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
NiCl ₂ 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.