

## 705. BACILLUS INFERNUS MEDIUM

NaCl	5.80	g
NH <sub>4</sub> Cl	1.00	g
K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O	0.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> 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 <sub>2</sub> 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% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Adjust pH to 7.5 with NaOH, dispense under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas. Prior to inoculation the pH of the complete medium should be checked and adjusted to 7.5, if necessary.

### *Trace element solution:*

Na <sub>2</sub> -EDTA x 2 H <sub>2</sub> O	0.50	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.15	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.10	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
ZnCl <sub>2</sub>	0.10	g
AlCl <sub>3</sub> x 6 H <sub>2</sub> O	40.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	40.00	mg
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	30.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	20.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	20.00	mg
H <sub>3</sub> BO <sub>3</sub>	10.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> 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.