

143. CLOSTRIDIUM SP. LA1 MEDIUM

Crotonic acid	6.00	g
NaOH	0.30	g
(NH ₄) ₂ HPO ₄	0.15	g
K ₂ HPO ₄	0.10	g
MgCl ₂ x 6 H ₂ O	0.03	g
CaCl ₂ x 2 H ₂ O	0.04	g
NH ₄ Cl	0.05	g
MgSO ₄ x 7 H ₂ O solution (0.1% w/v)	0.60	ml
MnSO ₄ x 2 H ₂ O solution (0.1% w/v)	0.40	ml
FeSO ₄ x 7 H ₂ O solution (0.1% w/v in 0.1 N H ₂ SO ₄)	0.40	ml
(NH ₄) ₆ Mo ₇ O ₂₄ x 4 H ₂ O	10.00	mg
Biotin solution (0.1% w/v)	0.04	ml
p-Aminobenzoic acid solution (0.1% w/v)	0.80	ml
Tryptone (BD BBL)	1.00	g
Yeast extract (OXOID)	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
K ₂ CO ₃	4.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except potassium carbonate) and sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. An anoxic stock solution of the potassium carbonate is autoclaved separately under 80% N₂ and 20% CO₂ gas mixture and thereafter added to the sterile medium. Adjust pH of the complete medium to 6.8 - 7.0. Prior to inoculation add 10 - 20 mg/l sodium dithionite from a 5% (w/v) stock solution that has been freshly prepared under 100% N₂ gas and sterilized by filtration to reduce the medium.