

52. CLOSTRIDIUM KLUYVERI MEDIUM (modified)

K-acetate	10.00	g
K ₂ HPO ₄	0.31	g
KH ₂ PO ₄	0.23	g
NH ₄ Cl	0.25	g
MgSO ₄ x 7 H ₂ O	0.20	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Yeast extract	1.00	g
Resazurin	0.50	mg
Ethanol	20.00	ml
NaHCO ₃	2.50	g
Seven vitamin solution (see medium 503)	1.00	ml
Cysteine-HCl x H ₂ O	0.25	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	1000.00	ml

Dissolve all ingredients (except ethanol, bicarbonate, reducing agents and vitamins) in distilled water and boil for 1 min. After cooling to room temperature under 80% N₂ and 20% CO₂ gas atmosphere, add ethanol, dispense in Hungate tubes and autoclave. After autoclaving, add bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere and vitamins from an anoxic and filter-sterilized stock solution. Before inoculation, add cysteine and sulfide from sterile anoxic stock solutions prepared under N₂. Adjust pH of medium to 6.8 – 7.0, if necessary. If reduction of medium is not complete after inoculation, add 10 – 20 mg/l sodium dithionite from a 5% w/v solution freshly prepared under N₂ and filter sterilized.