

## 52. CLOSTRIDIUM KLUYVERI MEDIUM

K-acetate	10.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.31	g
KH <sub>2</sub> PO <sub>4</sub>	0.23	g
NH <sub>4</sub> Cl	0.25	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> 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
Na-resazurin solution (0.1% w/v)	0.50	ml
Ethanol	20.00	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Seven vitamin solution (see medium 503)	1.00	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.25	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

Dissolve all ingredients (except ethanol, carbonate, vitamins, cysteine and sulfide) and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Add ethanol, then dispense medium under same gas atmosphere in Hungate-type tubes or serum vials and autoclave. After autoclaving, add carbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and vitamins from an anoxic and filter-sterilized stock solution prepared under 100% N<sub>2</sub> gas. Before inoculation, add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of medium to 6.8 – 7.0, if necessary.

*Note: 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 100% N<sub>2</sub> gas and filter sterilized.*