

## 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
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Ethanol absolute	20.00	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
<b>Seven vitamins solution</b>	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

1. 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.

2. 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.

### Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally

make up to 1000.00 ml.

### Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na <sub>2</sub> SeO <sub>3</sub> × 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> × 2 H <sub>2</sub> O	4.00	mg
Distilled water	1000.00	ml

### Seven vitamins solution (from medium 503)

Vitamin B <sub>12</sub>	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl × 2 H <sub>2</sub> O	200.00	mg
Distilled water	1000.00	ml