

## 710: THERMOANAEROBACTER (KoKo) MEDIUM

Tryptone (BD Bacto)	1.00	g
Peptone (meat)	1.00	g
Yeast extract (BD Bacto)	1.00	g
K <sub>2</sub> HPO <sub>4</sub>	1.60	g
NaH <sub>2</sub> PO <sub>4</sub> × 2 H <sub>2</sub> O	1.00	g
NH <sub>4</sub> Cl	0.50	g
MgSO <sub>4</sub> × 6 H <sub>2</sub> O	0.16	g
<b>Trace element solution SL-11</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
CaCl <sub>2</sub> × 2 H <sub>2</sub> O	0.06	g
NaHCO <sub>3</sub>	1.00	g
D-Glucose	5.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl × H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S × 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins, cysteine and sulfide), adjust pH to 7.0 and sparge medium with 100% N<sub>2</sub> 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. After sterilization add calcium chloride, glucose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N<sub>2</sub> gas atmosphere. Add bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Vitamins are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. The pH of the complete medium should be at 7.0.

2. Note: Supplementation of medium with 10.00 g/l MOPS buffer (pH 6.9 - 7.0; 10% (w/v) anoxic stock solution) may enhance the buffer capacity of the medium.

For DSM 12299: Omit D-glucose.

### Trace element solution SL-11 (from medium 722)

Na <sub>2</sub> -EDTA × 2 H <sub>2</sub> O	5.20	g
FeCl <sub>2</sub> × 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> × 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> × 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> × 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> × 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> × 2 H <sub>2</sub> O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 ml.

### Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml