

710: THERMOANAEROBACTER (KoKo) MEDIUM

This recipe contains strain-specific modifications for *Thermoanaerobacter siderophilus* DSM 12299 *

Final pH: 7.0

Final volume: 1002 ml

Tryptone (BD Bacto)	1.00	g
Peptone (meat)	1.00	g
Yeast extract (BD Bacto)	1.00	g
K ₂ HPO ₄	1.60	g
NaH ₂ PO ₄ x 2 H ₂ O	1.00	g
NH ₄ Cl	0.50	g
MgSO ₄ x 6 H ₂ O	0.16	g
Trace element solution SL-11	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
CaCl ₂ x 2 H ₂ O	0.06	g
NaHCO ₃	1.00	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ 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% 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. After sterilization add calcium chloride, glucose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Add bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins are prepared under 100% N₂ 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.

* Omit D-glucose.

Trace element solution SL-11 (from medium 722)

Na ₂ -EDTA x 2 H ₂ O	5.20	g
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg

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CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ 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 ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml