

## 1004: ANAEROLINEA MEDIUM

Final pH: 7.0

Final volume: 1003 ml

KH <sub>2</sub> PO <sub>4</sub>	0.14	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.20	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
NH <sub>4</sub> Cl	0.54	g
<b>Trace element solution SL-11</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	2.50	g
Yeast extract	2.30	g
D-Glucose	2.20	g
<b>Wolin's vitamin solution (10x)</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

Dissolve ingredients (except bicarbonate, yeast extract, vitamins, glucose, and reducing agents), then 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 solid bicarbonate and adjust pH to 6.4 - 6.8. Dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins (sterilized by filtration), glucose, sulfide, and cysteine from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust the pH of the complete medium to 7.0, if necessary.

For [DSM 16554](#), [DSM 16555](#), [DSM 17877](#): Replace glucose with 7.20 g/l sucrose and reduce amount of yeast extract to 0.10 g/l.

For [DSM 16556](#): Omit glucose and reduce amount of yeast extract to 0.10 g/l.

For [DSM 22659](#): Increase amount of glucose to 5.00 g/l.

For [DSM 23815](#): Replace glucose with 2.00 g/l cellobiose added after autoclaving from a sterile anoxic stock solution sterilized by filtration.

For [DSM 103421](#): Reduce amount of yeast extract to 0.1 g/l

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

Na <sub>2</sub> -EDTA x 2 H <sub>2</sub> O	5.20	g
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

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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	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.

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

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