1004: ANAEROLINEA MEDIUM

Dissolve ingredients (except bicarbonate, yeast extract, vitamins, glucose and reducing agents), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate and adjust pH to 7.0. Dispense medium under 80% N₂ and 20% CO₂ 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% N₂ gas. Adjust pH of 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)

- Na₂-EDTA x 2 H₂O: 5.20 g
- FeCl₂ x 4 H₂O: 1.50 g
- ZnCl₂: 70.00 mg
- MnCl₂ x 4 H₂O: 100.00 mg
- H₃BO₃: 6.00 mg
- CoCl₂ x 6 H₂O: 190.00 mg
- CuCl₂ x 2 H₂O: 2.00 mg
- NiCl₂ x 6 H₂O: 24.00 mg
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\[ \text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O} \quad 36.00 \quad \text{mg} \]
\[ \text{Distilled water} \quad 1000.00 \quad \text{ml} \]

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)

\[ \text{NaOH} \quad 0.50 \quad \text{g} \]
\[ \text{Na}_2\text{SeO}_3 \times 5\text{H}_2\text{O} \quad 3.00 \quad \text{mg} \]
\[ \text{Na}_3\text{WO}_4 \times 2\text{H}_2\text{O} \quad 4.00 \quad \text{mg} \]
\[ \text{Distilled water} \quad 1000.00 \quad \text{ml} \]

**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