

516: ANAEROCELLUM MEDIUM

NH ₄ Cl	0.33	g
KH ₂ PO ₄	0.33	g
KCl	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
Trace element solution SL-10	1.00	ml
Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Cellobiose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins, carbonate, cellobiose and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add vitamins, cellobiose and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Stock solutions of vitamins and cellobiose should be sterilized by filtration. The pH of the complete medium should be at 7.1 - 7.3.

For DSM 6724: Replace cellobiose with 5.00 g/l soluble starch as substrate added to the autoclaved medium from a sterile anoxic stock solution.

For DSM 9003: Supplement medium with 1.00 g/l Trypticase peptone and increase amount of yeast extract to 2.00 g/l.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
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
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	990.00	ml

516: ANAEROCELLUM MEDIUM

First dissolve FeCl_2 in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 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 ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml