

## 516. ANAEROCELLUM MEDIUM

NH <sub>4</sub> Cl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
KCl	0.33	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.33	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.33	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract	0.50	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Vitamin solution (see medium 141)	10.00	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Cellobiose	5.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins, carbonate, cellobiose and sulfide), 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. 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% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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.