

**1116. STEROIDOBACTER MEDIUM**

NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
KCl	0.50	g
NH <sub>4</sub> Cl	0.25	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
Na <sub>2</sub> SO <sub>4</sub>	0.07	g
NaNO <sub>3</sub>	0.42	g
Distilled water	1000.00	ml

For growth with testosterone, dissolve testosterone in acetone (20 mg/ml), dispense portions of the solution, e.g. 0.1 ml portions for 10 ml medium, to anaerobic culture tubes. Let the solvent evaporate to dryness. Dispense 10 ml portions of medium and stream with N<sub>2</sub>/CO<sub>2</sub> (80:20). Close the vessels and autoclave. Treat the vessels in an ultrasonic bath to detach and suspend the testosterone.

For growth with heptanoate, omit testosterone. Sterilize the gassed medium in anaerobic vessels by autoclaving. After cooling, add 325 mg/l heptanoic acid from a 20fold, pH adjusted, filter sterilized anaerobic stock solution.

After autoclaving, add (per 10 ml) 0.3 ml NaHCO<sub>3</sub>-solution (84 g/l, autoclaved under CO<sub>2</sub>-atmosphere), 1 µl trace element solution SL 10 (see below), 1 µl selenite tungstate solution (see below), and 1 µl vitamin solution (see below). Adjust pH to 7.2. Incubate the cultures in the dark for up to 4 weeks. Briefly shake the cultures once per day.

*Trace element solution SL-10:*

HCl (25%; 7.7 M)	10.00	ml
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
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	990.00	ml

*Selenite and tungstate solution:*

NaOH	0.5	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4	mg
Distilled water	1000	ml

*Vitamin solution:*

Vitamin B <sub>12</sub>	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine-HCl	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
$\alpha$ -lipoic acid	50.00	mg
p-aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H <sub>2</sub> O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.