862: STEROIDOBACTER MEDIUM

Final pH: 7.2
Final volume: 1000 ml

NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
KH_2PO_4	0.20	g
KCl	0.50	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.15	g
Na_2SO_4	0.07	g
$NaNO_3$	0.42	g
Distilled water	1000.00	ml

1. For growth with testosterone, dissolve testosterone in acetone (20 mg/ml) and 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 in the culture tubes and stream with N_2-CO_2 (80:20, v/v). Close the vessels and autoclave. Treat the vessels in an ultrasonic bath to detach and suspend the testosterone.

2. For growth with heptanoate, omit testosterone. Sterilize the gassed medium in anaerobic vessels by autoclaving. Add 2.5 mM heptanoate from a 20-fold filter-sterilized anaerobic stock solution after cooling.

3. After autoclaving, add (per 10 ml) 0.3 ml $NaHCO_3$ solution (84 g/L, autoclaved under a CO_2 atmosphere), 1 ï¿½l Trace element solution SL-10 (see Medium No. 433), 1 ï¿½l Selenite-tungstate solution (see below) and 1 ï¿½l 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.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Vitamin solution

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine hydrochloride	10.00	mg
Biotin	20.00	mg

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Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotinamide	25.00	mg
alpha-lipoic acid	50.00	mg
p-Aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml