1022: CRYPTOANAEROBACTER MEDIUM

4-Hydroxybenzoic acid	0.45	g
K_2HPO_4	0.40	g
NH_4Cl	0.40	g
Yeast extract (BD Bacto)	5.00	g
Casamino acids (BD Bacto)	1.00	g
Trace element solution SL-10	2.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
***C. sporogenes* supernatant**	350.00	ml
$MgCl_2$ x 6 H_2O	0.08	g
$CaCl_2$ x 2 H_2O	0.06	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	650.00	ml

1. Dissolve ingredients (except bicarbonate, C. sporogenes supernatant, magnesium chloride, calcium chloride, and vitamins), adjust pH to 7.0 - 7.5 and boil medium for 1 min, then cool to room temperature under 80% N_2 and 20% CO_2 gas mixture. Dissolve solid bicarbonate, adjust pH to 7.8, dispense the solution under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add the appropriate amount of sterile and anoxic supernatant of C. sporogenes and complete the medium by adding magnesium chloride, calcium chloride and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N_2 gas. The final pH of the medium should be 7.5 - 8.0.

2. It may be necessary to add 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 gas and filter-sterilized), if the medium is not completely reduced after inoculation.

3. Note: For transfers use 10% (v/v) inoculum.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg

Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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

C. sporogenes supernatant

Cultivate *Clostridium* sp. DSM 754 for 5 to 8 days at 37°C in the medium 1022, but omit 4-hydroxybenzoic acid, replace the *C. sporogenes* supernatant with distilled water and add after autoclaving 0.30 g/l Na_2S x 9 H_2O from a sterile anoxic stock solution prepared under 100% N_2 gas. Adjust pH of the complete medium to 7.0. Disrupt cells of the grown culture by autoclaving at 121°C for 20 min . Centrifuge autoclaved culture at 18000 x g for 20 min. Discard cell pellet and store the supernatant in screw capped bottles at -20°C . Before use sterilize the supernatant by autoclaving under 100% N_2 gas atmosphere in vials suitable for anaerobic cultivation.

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