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 (see medium 320)	2.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
C. sporogenes supernatant (see below)	350.00	ml
Vitamin solution (see medium 141)	10.00	ml
$MgCl_2$ x 6 H_2O	0.08	g
$CaCl_2$ x 2 H_2O	0.06	g
Distilled water	650.00	ml

Dissolve ingredients (except bicarbonate, *C. sporogenes* supernatant, vitamins, magnesium and calcium chloride), 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 vitamins (sterilized by filtration), magnesium and calcium chloride from sterile anoxic stock solutions prepared under 100% N_2 gas. The final pH of the medium should be 7.5 - 8.0. 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.
Note: For transfers use 10% (v/v) inoculum.

Supernatant of stationary culture of C. sporogenes:
Cultivate *Clostridium* sp. DSM 754 for 5 to 8 days at 37°C in the above medium, 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.