1086. DESULFATIRHABDIUM MEDIUM

Na_2HPO_4 x 2 H_2O	0.53	g
KH_2PO_4	0.41	g
NH_4Cl	0.30	g
$CaCl_2$ x 2 H_2O	0.11	g
$MgCl_2$ x 6 H_2O	0.10	g
NaCl	0.30	g
Na_2SO_4	2.80	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Yeast extract	0.02	g
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Vitamin solution (see medium 141)	10.00	ml
Na-crotonate solution (1 M, see medium 870)	20.00	ml
Na-benzoate	0.43	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, crotonate, benzoate and sulfide) and sparge medium with 80% N_2 and 20% CO_2 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. Add vitamins, crotonate, benzoate and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The crotonate and vitamin solutions should be sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2. After inoculation pressurize the vessels with sterile 80% N_2 and 20% CO_2 gas mixture to 0.7 bar overpressure.