311. SPOROMUSA MEDIUM (BETAINE)

NH_4Cl	0.50	g
$MgSO_4$ x 7 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.25	g
NaCl	2.25	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Yeast extract	2.00	g
Casitone	2.00	g
Betaine x H_2O	6.70	g
Na-resazurin solution (0.1% w/v)	0.50	ml
K_2HPO_4	0.35	g
KH_2PO_4	0.23	g
Na_2CO_3	1.00	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphates, carbonate, vitamins, cysteine 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 phosphates, vitamins (sterilized by filtration), cysteine and sulfide to the medium after autoclaving from sterile stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to pH 7.0, if necessary.