

311. SPOROMUSA MEDIUM (BETAINE)

NH ₄ Cl	0.50	g
MgSO ₄ x 7 H ₂ O	0.50	g
CaCl ₂ x 2 H ₂ O	0.25	g
NaCl	2.25	g
FeSO ₄ x 7 H ₂ O solution (0.1% w/v in 0.1 N H ₂ SO 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 ₂ O	6.70	g
Na-resazurin solution (0.1% w/v)	0.50	ml
K ₂ HPO ₄	0.35	g
KH ₂ PO ₄	0.23	g
NaHCO ₃	4.00	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphates, bicarbonate, vitamins, cysteine and sulfide) and sparge medium with 80% N₂ and 20% CO₂ 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% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to pH 7.0, if necessary.