323. TREPONEMA SACCHAROPHILUM MEDIUM

$CaCl_2$ x 2 H_2O	0.12	g
$MgSO_4$ x 7 H_2O	0.19	g
KH_2PO_4	0.45	g
K_2HPO_4	0.45	g
NaCl	0.90	g
$(NH_4)_2SO_4$	0.90	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
n-Butyric acid	0.40	ml
iso-Butyric acid	0.40	ml
DL-2-Methylbutyric acid	0.20	ml
n-Valeric acid	0.20	ml
iso-Valeric acid	0.20	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
L-Cysteine-HCl x H_2O	1.00	g
Na_2CO_3	2.50	g
D-Glucose	2.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, carbonate and glucose), adjust pH to 7.0 and sparge medium with 100% CO_2 gas for 30 – 45 min to make it anoxic. Add the cysteine and carbonate, then equilibrate the medium with the CO_2 gas to pH 7.0. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add glucose from an anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.
For solid medium add 12.00 g/l agar (BD Bacto).