323. TREPONEMA SACCHAROPHILUM MEDIUM

CaCl₂ x 2 H₂O 0.12 g
MgSO₄ x 7 H₂O 0.19 g
KH₂PO₄ 0.45 g
K₂HPO₄ 0.45 g
NaCl 0.90 g
(NH₄)₂SO₄ 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 H₂O 1.00 g
Na₂CO₃ 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% CO₂ gas for 30 – 45 min to make it anoxic. Add the cysteine and carbonate, then equilibrate the medium with the CO₂ 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% N₂ 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).