

275. TREPONEMA SUCCINIFACIENS MEDIUM**Solution A:**

CaCl ₂ x 2 H ₂ O	0.1	g
MgSO ₄ x 7 H ₂ O	0.1	g
KH ₂ PO ₄	0.5	g
K ₂ HPO ₄	0.5	g
NaCl	1.0	g
Rumen fluid, clarified (see medium 1310)	300.0	ml
Yeast extract (OXOID)	0.5	g
Peptone (BD BACTO)	0.5	g
(NH ₄) ₂ SO ₄	0.5	g
Na-resazurin solution (0.1% w/v)	0.5	ml
L-Cysteine-HCl x H ₂ O	0.5	g
Distilled water	580.0	ml

Solution B:

Glucose	10.0	g
Distilled water	50.0	ml

Solution C:

1 M K-phosphate buffer, pH 7.4 20.0 ml

Combine 16.04 ml of 1 M K₂HPO₄ with 3.96 ml of 1 M KH₂PO₄ stock solution to reach a pH of around 7.4.

Solution D:

NaHCO ₃	2.5	g
Distilled water	50.0	ml

Dissolve ingredients of *solution A*, adjust pH to 6.2 – 6.3 and sparge with 100% N₂ gas atmosphere to make it anoxic. Then distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. *Solutions B* and *C* are prepared under 100% N₂ gas atmosphere and autoclaved. *Solution D* is prepared under 80% N₂ and 20% CO₂ gas atmosphere and autoclaved. To complete the medium appropriate amounts of *solutions B* to *D* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be at 7.4.