

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

CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.1	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.1	g
KH <sub>2</sub> PO <sub>4</sub>	0.5	g
K <sub>2</sub> HPO <sub>4</sub>	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 <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.5	g
Na-resazurin solution (0.1% w/v)	0.5	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.5	g
Distilled water	600.0	ml

**Solution B:**

Glucose	10.0	g
Distilled water	50.0	ml

**Solution C:**

Na <sub>2</sub> CO <sub>3</sub>	1.5	g
Distilled water	30.0	ml

Dissolve ingredients of *solution A* and sparge with 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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* is prepared under 100% N<sub>2</sub> gas atmosphere and autoclaved. *Solution C* is prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and autoclaved. To complete the medium appropriate amounts of *solutions B* and *C* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be at 7.4.