

**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:**

1 M K-phosphate buffer, pH 7.4	20.0	ml
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Combine 16.04 ml of 1 M K<sub>2</sub>HPO<sub>4</sub> with 3.96 ml of 1 M KH<sub>2</sub>PO<sub>4</sub> stock solution to reach a pH of around 7.4.

**Solution D:**

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

Dissolve ingredients of *solution A*, adjust pH to 6.2 – 6.3 and sparge with 100% N<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* and *C* are prepared under 100% N<sub>2</sub> gas atmosphere and autoclaved. *Solution D* 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* to *D* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be at 7.4.