

275: TREPONEMA SUCCINIFACIENS MEDIUM

Solution A	920.00	ml
Solution B	50.00	ml
Solution C	30.00	ml

Dissolve ingredients of solution A and sparge with 80% N₂ and 20% CO₂ 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% N₂ gas atmosphere and autoclaved. Solution C is prepared under 80% N₂ and 20% CO₂ 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.

Solution A

CaCl ₂ x 2 H ₂ O	0.10	g
MgSO ₄ x 7 H ₂ O	0.10	g
KH ₂ PO ₄	0.50	g
K ₂ HPO ₄	0.50	g
NaCl	1.00	g
Clarified rumen fluid	300.00	ml
Yeast extract (OXOID)	0.50	g
Peptone (BD BACTO)	0.50	g
(NH ₄) ₂ SO ₄	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
Distilled water	620.00	ml

Solution B

Glucose	10.00	g
Distilled water	50.00	ml

Solution C

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g

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for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.