212a. TREPONEMA RUMINIS MEDIUM

Solution A:		
KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.33	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Rumen fluid, clarified (see medium 1310)	50.00	ml
Trypticase peptone (BD BBL)	1.00	g
Na_2SO_4	2.80	g
Vitamin K_1 solution (see medium 78)	1.00	ml
Volatile fatty acid mixture (see medium 330)	3.10	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	860.00	ml
Solution B:		
Na_2CO_3	1.50	g
Distilled water	30.00	ml
Solution C:		
Soluble starch	0.40	g
Distilled water	4.00	ml
Solution D:		
D-Glucose	0.80	g
Distilled water	4.00	ml
Solution E:		
Sugar mix (see medium 843)	30.00	ml
Solution F:		
Vitamin solution (see medium 503)	1.00	ml
Solution G:		
Na-pyruvate	0.50	g
Distilled water	2.00	ml

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Solution H:

L-Cysteine-HCl x H_2O	0.30	g
Distilled water	10.00	ml

Solution I:

DL-Dithiothreitol (DTT)	0.40	g
Distilled water	10.00	ml

Add and dissolve ingredients of *solution A,* adjust pH to 7.0 and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. *Solution B* is prepared under 80% N_2 and 20% CO_2 gas atmosphere and autoclaved. *Solutions C, D* and *H* are autoclaved under 100% N_2 gas atmosphere. *Solutions E, F*, *G* and *I* are prepared under 100% N_2 gas and sterilized by filtration. To complete the medium appropriate amounts of *solutions B* to *I* are added to the sterile *solution A* in the sequence as indicated.
Note: Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days. After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.