# 212a. TREPONEMA RUMINIS MEDIUM

**Solution A:**
- KH₂PO₄ 0.50 g
- MgCl₂ x 6 H₂O 0.33 g
- NaCl 0.40 g
- NH₄Cl 0.40 g
- CaCl₂ x 2 H₂O 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
- Na₂SO₄ 2.80 g
- Vitamin K₁ 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:**
- Na₂CO₃ 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 H₂O 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% N₂ and 20% CO₂ 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% N₂ and 20% CO₂ gas atmosphere and autoclaved. Solutions C, D and H are autoclaved under 100% N₂ gas atmosphere. Solutions E, F, G and I are prepared under 100% N₂ 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.