212a: TREPONEMA RECTALE MEDIUM

**Solution A** 917.00 ml  
**Solution B** 30.00 ml  
**Solution C** 4.00 ml  
**Solution D** 4.00 ml  
**Solution E** 30.00 ml  
**Solution F** 1.00 ml  
**Solution G** 2.00 ml  
**Solution H** 10.00 ml  
**Solution I** 10.00 ml

1. 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 to 30% of their volume and autoclave. Solution B is prepared under 80% N₂ and 20% CO₂ gas atmosphere and autoclaved. Solutions C, D, and H are autoclaved under a 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.

2. 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.

For **DSM 103462**: Replace the sugar mix of solution E with the sugar mix of medium 110a. Omit solution C, solution D, and solution H. After inoculation, add a sterile gas mixture of 80% H₂ and 20% CO₂ to an overpressure of 0.5 bar.

### 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** 1.00 ml  
- **Selenite-tungstate solution** 1.00 ml  
- **Clarified rumen fluid** 50.00 ml  
- Trypticase peptone (BD BBL) 1.00 g  
- Na₂SO₄ 2.80 g
### Microorganisms

#### 212a: TREPONEMA RECTALE MEDIUM

**Vitamin K<sub>1</sub> solution**
- 1.00 ml

**Volatile fatty acid mixture**
- 3.10 ml

**Sodium resazurin (0.1% w/v)**
- 0.50 ml

**Distilled water**
- 860.00 ml

**Solution B**
- Na<sub>2</sub>CO<sub>3</sub> 1.50 g
- Distilled water 30.00 ml

**Solution C**
- Starch (soluble) 0.40 g
- Distilled water 4.00 ml

**Solution D**
- D-Glucose 0.80 g
- Distilled water 4.00 ml

**Solution E**
- Sugar mix 30.00 ml

**Solution F**
- Seven vitamins solution 1.00 ml

**Solution G**
- Na-pyruvate 0.50 g
- Distilled water 2.00 ml

**Solution H**
- L-Cysteine HCl x H<sub>2</sub>O 0.30 g
- Distilled water 10.00 ml

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

**Trace element solution SL-10** (from medium 320)
- HCl (25%) 10.00 ml
- FeCl<sub>2</sub> x 4 H<sub>2</sub>O 1.50 g

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Microorganisms
212a: TREPONEMA RECTALE MEDIUM

ZnCl$_2$ 70.00 mg
MnCl$_2$ x 4 H$_2$O 100.00 mg
H$_3$BO$_3$ 6.00 mg
CoCl$_2$ x 6 H$_2$O 190.00 mg
CuCl$_2$ x 2 H$_2$O 2.00 mg
NiCl$_2$ x 6 H$_2$O 24.00 mg
Na$_2$MoO$_4$ x 2 H$_2$O 36.00 mg
Distilled water 990.00 ml

First dissolve FeCl$_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)
NaOH 0.50 g
Na$_2$SeO$_3$ x 5 H$_2$O 3.00 mg
Na$_2$WO$_4$ x 2 H$_2$O 4.00 mg
Distilled water 1000.00 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 for 20 min. The supernatant is made anoxic by sparging with 100% N$_2$ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Vitamin K$_1$ solution (from medium 78)
Vitamin K$_1$ 0.10 ml
Ethanol (95 %) 20.00 ml

Dissolve 0.1 ml of vitamin K$_1$ in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Volatile fatty acid mixture (from medium 330)
Acetic acid 548.50 ml
Propionic acid 193.50 ml
Butyric acid 129.00 ml
n-Valeric acid 32.25 ml
iso-Butyric acid 32.25 ml
DL-2-Methylbutyric acid 32.25 ml
iso-Valeric acid 32.25 ml
Sugar mix (from medium 843)

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<th>Amount</th>
<th>Unit</th>
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<tr>
<td>Cellobiose</td>
<td>56.70 g</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>56.70 g</td>
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</tr>
<tr>
<td>Maltose x H₂O</td>
<td>60.00 g</td>
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<tr>
<td>Trehalose</td>
<td>63.30 g</td>
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<tr>
<td>D-Xylose</td>
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<tr>
<td>Distilled water</td>
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Seven vitamins solution (from medium 503)

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<tr>
<td>Vitamin B₁₂</td>
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<td>D-(+)-biotin</td>
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<tr>
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<td>Calcium pantothenate</td>
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<td>Pyridoxine hydrochloride</td>
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<td>Thiamine-HCl x 2 H₂O</td>
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