

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

KH ₂ PO ₄	0.50	g
MgCl ₂ x 6 H ₂ O	0.33	g
NaCl	0.40	g
NH ₄ Cl	0.40	g
CaCl ₂ x 2 H ₂ O	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
Na ₂ SO ₄	2.80	g

212a: TREPONEMA RECTALE MEDIUM

Vitamin K₁ 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 ₂ CO ₃	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 ₂ 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 ₂ x 4 H ₂ O	1.50	g

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 FeCl₂ 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% 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.

Vitamin K₁ solution (from medium 78)

Vitamin K ₁	0.10	ml
Ethanol (95 %)	20.00	ml

Dissolve 0.1 ml of vitamin K₁ 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

212a: TREPONEMA RECTALE MEDIUM

Sugar mix (from medium 843)

Cellobiose	56.70	g
Sucrose	56.70	g
Maltose x H ₂ O	60.00	g
Trehalose	63.30	g
D-Xylose	25.00	g
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B ₁₂	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H ₂ O	200.00	mg
Distilled water	1000.00	ml