

912: TREPONEMA R8 MEDIUM

NaCl	1.00	g
MgCl ₂ x 6 H ₂ O	0.50	g
KH ₂ PO ₄	0.20	g
K ₂ HPO ₄	0.25	g
NH ₄ Cl	0.30	g
KCl	0.30	g
CaCl ₂ x 2 H ₂ O	15.00	mg
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
MOPS (SIGMA)	2.10	g
D-Glucose	0.90	g
Yeast extract	0.19	g
Peptone (BD Bacto)	0.19	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	2.50	g
Clarified rumen fluid	50.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.40	g
Na ₂ S x 9 H ₂ O	0.40	g
Distilled water	950.00	ml

Dissolve ingredients (except bicarbonate, rumen fluid, vitamins, cysteine and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate, adjust pH to 7.2, distribute medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add rumen fluid, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere. Vitamins should be sterilized by filtration. Check pH of complete medium and adjust to 7.2, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
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

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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
$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$	3.00	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	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 .

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml