

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 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
MOPS (SIGMA)	2.10	g
D-Glucose	0.90	g
Yeast extract	0.19	g
Peptone (BD Bacto)	0.19	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	2.50	g
Rumen fluid, clarified (see medium 1310)	50.00	ml
Vitamin solution (see medium 141)	10.00	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.