

169b. TREPONEMA AZOTONUTRICIUM MEDIUM**Solution A:**

CaCl ₂ x 2 H ₂ O	0.04	g
MgSO ₄ x 7 H ₂ O	0.50	g
Yeast extract (OXOID)	4.00	g
D-Glucose	4.00	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
L-Cysteine-HCl x H ₂ O	0.50	g
Distilled water	950.00	ml

Solution B:

NaHCO ₃	1.00	g
Distilled water	20.00	ml

Solution C:

0.5 M K-phosphate buffer, pH 7.4	20.00	ml
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Combine 16.04 ml of 0.5 M K₂HPO₄ with 3.96 ml of 0.5 M KH₂PO₄ stock solution to reach a pH of around 7.4.

Solution D:

Vitamins solution (see medium 503)	1.00	ml
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Solution E:

DL-Dithiothreitol	0.15	g
Distilled water	10.00	ml

Dissolve ingredients of *solution A* (except cysteine) and sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Add cysteine and adjust pH 7.2 with KOH, then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. *Solution B* is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere, *solution C* is autoclaved under 100% N₂ gas atmosphere and *solutions D* and *E* are sterilized by filtration under 100% N₂ gas atmosphere. To complete the medium appropriate amounts of *solutions B, C, D* and *E* are added to the sterile *solution A* in the sequence as indicated. Final pH of the complete medium should be 7.5.