

509. SPIROCHAETA THERMOPHILA MEDIUM

Solution A:

NaCl	15.00	g
MgCl ₂ x 6 H ₂ O	2.30	g
KCl	0.50	g
NH ₄ Cl	0.30	g
KH ₂ PO ₄	0.20	g
CaCl ₂ x 2 H ₂ O	0.03	g
Selenite-tungstate solution (see medium 385)	1.00	ml
Yeast extract	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	920.00	ml

Solution B:

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C:

Trace element solution SL-10 (see medium 320)	1.00	ml
---	------	----

Solution D:

Vitamins solution (see medium 141)	10.00	ml
------------------------------------	-------	----

Solution E:

Starch	1.00	g
Distilled water	10.00	ml

Solution F:

Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	10.00	ml

Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture to reach a pH below 6 (at least 30 -45 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. *Solution B* is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. *Solutions C, E* and *F* are autoclaved under 100% N₂ gas atmosphere. *Solution D* is prepared under 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of *solutions B* to *F* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be 7.2 – 7.4.