

## 509a. SPIROCHAETA RI 19.B1 MEDIUM

### Solution A:

NaCl	4.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.80	g
KCl	0.50	g
NH <sub>4</sub> Cl	0.30	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.03	g
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

### Solution B:

Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Distilled water	20.00	ml

### Solution C:

Trace element solution SL-10 (see medium 320)	1.00	ml
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### Solution D:

Vitamins solution (see medium 141)	10.00	ml
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### Solution E:

Starch	1.00	g
Distilled water	10.00	ml

### Solution F:

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	10.00	ml

*Solution A* is sparged with 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. *Solutions C, E* and *F* are autoclaved under 100% N<sub>2</sub> gas atmosphere. *Solution D* is prepared under 100% N<sub>2</sub> 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 6.8 – 7.0.