

## 509a: SPIROCHAETA RI 19.B1 MEDIUM

<b>Solution A</b>	962.00	ml
<b>Solution B</b>	20.00	ml
<b>Solution C</b>	1.00	ml
<b>Solution D</b>	10.00	ml
<b>Solution E</b>	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.

### 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
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	960.00	ml

### Solution B

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

### Solution C

<b>Wolin's vitamin solution (10x)</b>	1.00	ml
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### Solution D

Starch	1.00	g
Distilled water	10.00	ml

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### Solution E

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

### Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> 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
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4.00	mg
Distilled water	1000.00	ml

### 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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml