

## 503c. AMMONIFEX THIOPHILUS MEDIUM

### Solution A:

KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.25	g
NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

### Solution B:

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

Vitamins solution (see medium 141)	1.00	ml
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### Solution D:

Selenite-tungstate solution (see medium 385)	1.00	ml
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### Solution E:

Na <sub>2</sub> CO <sub>3</sub>	2.50	g
Distilled water	50.00	ml

### Solution F:

Na-formate	2.00	g
Distilled water	10.00	ml

### Solution G:

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	2.00	g
Distilled water	10.00	ml

### Solution H:

Na <sub>2</sub> S x 9 H <sub>2</sub> O, 3% w/v solution	0.30	g
Distilled water	10.00	ml

Sparge *solution A* with 100% CO<sub>2</sub> gas for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave.

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*Solutions B, D, F and H* are autoclaved separately under 100% N<sub>2</sub> gas. *Solutions C and G* are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. *Solution E* is autoclaved under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. To complete the medium appropriate amounts of *solutions B to H* are added to the sterile *solution A* in the sequence as indicated. Adjust pH of complete medium to 6.8, if necessary.