

## 795: STETTERIA MEDIUM

<b>Synthetic seawater (2 x conc.)</b>	500.00	ml
NaHCO <sub>3</sub>	0.16	g
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
<b>Modified Wolin's mineral solution</b>	15.00	ml
NiCl <sub>2</sub> x 6 H <sub>2</sub> O (0.1% w/v)	3.00	ml
Peptone (BD Bacto)	2.00	g
Yeast extract (OXOID)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered), powdered	10.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	500.00	ml

Dissolve ingredients except sulfur and sulfide, then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum vials (e.g., 20 ml medium per 100 ml bottle) that contain already the appropriate amount of sulfur. After closing pressurize bottles to 2 bar overpressure using 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture, then heat at 100°C for 1.5 hours on each of 3 successive days. Before use add sulfide from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere. Adjust pH of complete medium to 6.0, if necessary. Incubate without shaking.

For [DSM 25542](#), [DSM 25543](#): Use medium without adding 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture before sterilization and adjust pH of complete medium to 6.8.

### **Modified Wolin's mineral solution** (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.40	mg
Distilled water	1000.00	ml

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First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

### **Synthetic seawater (2 x conc.)** (from medium 795)

NaCl	55.40	g
SrCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	14.00	g
KI	0.10	mg
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	11.00	g
Na <sub>3</sub> -citrate	20.00	mg
KCl	1.30	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.50	g
NaBr	0.20	g
H <sub>3</sub> BO <sub>3</sub>	0.06	g
Distilled water	1000.00	ml