

**399a: ARCHAEOGLOBUS NEPTUNIUS MEDIUM**

|   |         |    |
|---|---------|----|
| KCl   | 0.34    | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O  | 4.00    | g  |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O  | 3.45    | g  |
| NH <sub>4</sub> Cl  | 0.25    | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O  | 0.14    | g  |
| K <sub>2</sub> HPO <sub>4</sub>   | 0.14    | g  |
| NaCl  | 18.00   | g  |
| Yeast extract (OXOID)   | 0.50    | g  |
| Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 7 H <sub>2</sub> O (0.1% w/v) | 2.00    | ml |
| <b>Modified Wolin's mineral solution</b>  | 10.00   | ml |
| Sodium resazurin (0.1% w/v)   | 0.50    | ml |
| NaHCO <sub>3</sub>  | 3.00    | g  |
| Na-pyruvate   | 1.00    | g  |
| Na <sub>2</sub> SO <sub>3</sub>   | 0.60    | g  |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O  | 0.50    | g  |
| Distilled water   | 1000.00 | ml |

Dissolve ingredients except bicarbonate, pyruvate, sulfite 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. Add bicarbonate and adjust pH to 6.5, then dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into serum vials (e.g., 20 ml per 100 ml bottle), seal and autoclave. Before use add pyruvate, sulfite and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Stock solutions of pyruvate and sulfide should be sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.

**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.