



Main sol. 1024

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| NaNO ₃ | 1.00 | g |
| NaHCO ₃ | 1.00 | g |
| Na ₂ S ₂ O ₃ x 5 H ₂ O | 1.00 | g |
| Sulfur powder | 3.00 | g |
| Wolin's vitamin solution | 10.00 | ml |
| Modified Wolin's mineral solution II | 10.00 | ml |
| Synthetic seawater | 1000.00 | ml |

1. Prepare the medium under an atmosphere of H₂/CO₂ (80:20) without adding the vitamins and NaHCO₃ in serum bottles and seal the serum tubes with butyl rubber stoppers. Steam medium for 3 hours on each of 3 successive days. To the sterile medium add, from filter sterilised stock solutions, the NaHCO₃ and vitamin solution.
2. Increase the 80% H₂ + 20% CO₂ gas phase pressure to 300 kPa. The final pH is 7.0.