

611. PYROBACULUM (BS) MEDIUM

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|--|--------|----|
| Synthetic seawater mix (see below) | 125.00 | ml |
| Trace element solution (see medium 141) | 10.00 | ml |
| $(\text{NH}_4)_2(\text{Fe}(\text{SO}_4)_2 \times 6 \text{H}_2\text{O})$ solution (0.01% w/v) | 2.00 | ml |
| NH_4Cl | 0.25 | g |
| KH_2PO_4 | 0.07 | g |
| Na-resazurin solution (0.1% w/v) | 0.50 | ml |
| KNO_3 | 1.00 | g |
| Yeast extract | 0.50 | g |
| NaHCO_3 | 2.00 | g |
| Distilled water | 875.00 | ml |

Dissolve ingredients (except nitrate, yeast extract and bicarbonate) and sparge medium with 100% N_2 gas for 30 – 45 min to make it anoxic. Adjust pH to 7.0 and dispense under 100% N_2 gas atmosphere into serum vials (e.g., 20 ml medium per 100 ml bottle). Replace gas phase with 80% H_2 and 20% CO_2 gas mixture and autoclave. Prior to inoculation add nitrate and yeast extract from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.0 – 7.2.

Synthetic seawater mix:

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|---|---------|---------------|
| NaCl | 47.15 | g |
| $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ | 18.10 | g |
| $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ | 7.00 | g |
| $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ | 3.13 | g |
| Na_2SO_4 | 3.24 | g |
| KCl | 1.20 | g |
| Na_2CO_3 | 0.10 | g |
| NaBr | 0.10 | g |
| KBr | 80.00 | mg |
| $\text{SrCl}_2 \times 6 \text{H}_2\text{O}$ | 72.00 | mg |
| H_3BO_3 | 52.00 | mg |
| Na_2HPO_4 | 8.10 | mg |
| NaF | 2.40 | mg |
| Na-silicate | 0.40 | mg |
| KI | 50.00 | μg |
| Distilled water | 1000.00 | ml |