169: TERETINEMA MEDIUM

<table>
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<th>Solution</th>
<th>Volume (ml)</th>
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<tbody>
<tr>
<td>Solution A</td>
<td>960.00</td>
</tr>
<tr>
<td>Solution B</td>
<td>20.00</td>
</tr>
<tr>
<td>Solution C</td>
<td>20.00</td>
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1. Dissolve ingredients of solution A (except cysteine) and sparge medium with 100% \( \text{N}_2 \) gas for 30 - 45 min to make it anoxic. Add cysteine and adjust pH 7.2 with KOH, then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% \( \text{N}_2 \) and 20% \( \text{CO}_2 \) gas atmosphere and solution C is autoclaved under 100% \( \text{N}_2 \) gas atmosphere. To complete the medium appropriate amounts of solutions B and C are added to the sterile solution A in the sequence as indicated. Final pH of the complete medium should be 7.5.

2. For solid medium add 10.00 g/l agar (BD Bacto) to solution A.

**Solution A**

- \( \text{CaCl}_2 \times 2 \text{H}_2\text{O} \) 0.04 g
- \( \text{MgSO}_4 \times 7 \text{H}_2\text{O} \) 0.50 g
- Yeast extract (OXOID) 4.00 g
- D-Glucose 2.00 g
- Sodium resazurin (0.1% w/v) 0.50 ml
- L-Cysteine HCl \( \times \text{H}_2\text{O} \) 0.50 g
- Agar, for solid medium (optional) 10.00 g
- Distilled water 960.00 ml

**Solution B**

- \( \text{NaHCO}_3 \) 1.00 g
- Distilled water 20.00 ml

**Solution C**

- \( \text{K}_2\text{HPO}_4 \) (0.5 M) 16.04 ml
- \( \text{KH}_2\text{PO}_4 \) (0.5 M) 3.96 ml

Combine 16.04 ml of 0.5 M \( \text{K}_2\text{HPO}_4 \) with 3.96 ml of 0.5 M \( \text{KH}_2\text{PO}_4 \) stock solution to reach a pH of around 7.4.