## Main sol. 397

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|---|---|---|
| **Solution A** | 882.00 | ml |
| **Solution B** | 30.00 | ml |
| **Solution D** | 1.00 | ml |
| **Solution E** | 10.00 | ml |
| **Solution F** | 10.00 | ml |
| Sodium ferulate | 10.00 | mM |

1. Add and dissolve ingredients of solution A and sparge medium with 80% $N_2$ and 20% $CO_2$ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Solution B is prepared under 80% $N_2$ and 20% $CO_2$ gas atmosphere and autoclaved. Solutions E and F are autoclaved under 100% $N_2$ gas atmosphere.

2. Solution D is prepared under 100% $N_2$ gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated.

3. Note: Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days . After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.

4. Add sodium ferulate to 10 mM final concentration from filter-sterilized anaerobic stock solution.