

## 710. THERMOANAEROBACTER (KoKo) MEDIUM

|   |         |    |
|---|---------|----|
| Tryptone (BD Bacto)                                   | 1.00    | g  |
| Peptone (meat)  | 1.00    | g  |
| Yeast extract (BD Bacto)                              | 1.00    | g  |
| K <sub>2</sub> HPO <sub>4</sub>                       | 1.60    | g  |
| NaH <sub>2</sub> PO <sub>4</sub> x 2 H <sub>2</sub> O | 1.00    | g  |
| NH <sub>4</sub> Cl                                    | 0.50    | g  |
| MgSO <sub>4</sub> x 6 H <sub>2</sub> O                | 0.16    | g  |
| Trace element solution SL-11 (see medium 722)         | 1.00    | ml |
| Na-resazurin solution (0.1% w/v)                      | 0.50    | ml |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O                | 0.06    | g  |
| NaHCO <sub>3</sub>                                    | 1.00    | g  |
| D-Glucose   | 5.00    | g  |
| Vitamin solution (see medium 141)                     | 10.00   | ml |
| L-Cysteine-HCl x H <sub>2</sub> O                     | 0.30    | g  |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O                | 0.30    | g  |
| Distilled water                                       | 1000.00 | ml |

Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins, cysteine and sulfide), adjust pH to 7.0 and sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add calcium chloride, glucose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N<sub>2</sub> gas atmosphere. Add bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Vitamins are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. The pH of the complete medium should be at 7.0.

*Note: Supplementation of medium with 10.00 g/l MOPS buffer (pH 6.9 - 7.0; 10% (w/v) anoxic stock solution) may enhance the buffer capacity of the medium.*

For DSM 12299 omit D-glucose.