

**1028. THERMINCOLA MEDIUM (CO)**

|  |         |    |
|--|---------|----|
| NH <sub>4</sub> Cl                       | 1.00    | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O   | 0.33    | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O   | 0.10    | g  |
| KCl                                      | 0.33    | g  |
| KH <sub>2</sub> PO <sub>4</sub>          | 0.50    | g  |
| Wolfe's mineral elixier (see medium 792) | 1.00    | ml |
| Na-acetate                               | 0.20    | g  |
| Na-resazurin solution (0.1% w/v)         | 0.50    | ml |
| NaHCO <sub>3</sub>                       | 0.50    | g  |
| Na <sub>2</sub> CO <sub>3</sub>          | 0.50    | g  |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O   | 1.00    | g  |
| Vitamin solution (see medium 141)        | 20.00   | ml |
| Yeast extract                            | 0.20    | g  |
| Distilled water                          | 1000.00 | ml |

Dissolve ingredients except carbonates, sulfide, vitamins and yeast extract, then sparge medium with 100% N<sub>2</sub> gas for 30 – 45 min to make it anoxic. Add carbonates and sulfide to the medium, dispense under 100% **carbon monoxide** gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 10 ml medium in 50 ml bottles) and autoclave. Prior to inoculation add vitamins (sterilized by filtration) and yeast extract from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. If the medium is not completely reduced after autoclaving (remains pink) add 0.30 g/l sodium sulfide from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere. Adjust pH of complete medium to 8.0 with a sterile anoxic solution of 1 N HCl.