



## Main sol. 1526b

|  |         |    |
|--|---------|----|
| Sea Salt (SIGMA)                       | 60.00   | g  |
| <b>Wolfe's mineral elixir</b>          | 1.00    | ml |
| Trypticase peptone (BD BBL)            | 1.00    | g  |
| Yeast extract (OXOID)                  | 1.00    | g  |
| Glucose                                | 1.00    | g  |
| Sodium resazurin (0.1% w/v)            | 0.50    | ml |
| Na <sub>2</sub> CO <sub>3</sub>        | 1.00    | g  |
| <b>Wolin's vitamin solution (10x)</b>  | 1.00    | ml |
| L-Cysteine HCl x H <sub>2</sub> O      | 0.50    | g  |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O | 0.50    | g  |
| Distilled water                        | 1000.00 | ml |

Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.