

**Main sol. 760****Main sol. 514**

1000.00 ml

1. Prepare liquid Bacto Marine Broth (see medium 514) and filter through normal filter paper. An iron sediment will collect in the filter. Boil the medium in a water bath and cool under an anaerobic gas mixture (see strain dependant modifications).
2. Dispense the medium into Hungate tubes or serum bottles containing finely divided sulphur (0.5% w/v), seal the tubes or bottles under the same anaerobic gas used when cooling the medium. Sterilise the medium at 100°C for 3 hours on 3 consecutive days. Reduce the medium by adding 10% neutralised sodium sulphide to a final concentration of 0.05 %. The medium should not give a heavy black precipitate, if it does the iron sediment was not adequately removed by filtering in the initial stages and the medium should be made again, making sure that the iron is removed by filtering.