

## Main sol. 1131

**Modified MJ synthetic seawater** 1000.00 ml

1. Sparge medium with 100% N<sub>2</sub> gas mixture for 10 min to make anoxic.
2. Adjust pH to 7.0 with NaOH.
3. Sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 10 min.

Sulfur (powder) 3.00 g/l

4. Dispense the medium (10 mL) into 100 mL serum bottles, containing 0,03g sulfur (powder) under a stream of H<sub>2</sub>/CO<sub>2</sub> (80:20) and seal with butyl rubber stoppers.

5. Autoclave at 121 °C for 20 min.

6. Prior to inoculation, add vitamins, NaNO<sub>3</sub>, and NaHCO<sub>3</sub> (from filter sterilized stock solutions under a N<sub>2</sub> gas atmosphere) to the medium.

**Vitamin solution** 10.00 ml/l

NaNO<sub>3</sub> 1.00 g/l

NaHCO<sub>3</sub> 1.00 g/l