

## 760: MARINE THERMOCOCCUS MEDIUM

This recipe contains strain-specific modifications for *Thermococcus stetteri* DSM 5262 \*

Final pH: \* 6.5

Final volume: 1000 ml

**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.

\* Incubate with a gas phase of 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Adjust pH of final medium to 6.5.

### Main sol. 514 (from medium 514)

Bacto peptone	5.00	g
Yeast extract (Bacto )	1.00	g
Fe(III) citrate	0.10	g
NaCl	19.45	g
MgCl <sub>2</sub> (anhydrous)	5.90	g
Na <sub>2</sub> SO <sub>4</sub>	3.24	g
CaCl <sub>2</sub>	1.80	g
KCl	0.55	g
NaHCO <sub>3</sub>	0.16	g
KBr	0.08	g
SrCl <sub>2</sub>	34.00	mg
H <sub>3</sub> BO <sub>3</sub>	22.00	mg
Na-silicate	4.00	mg
NaF	2.40	mg
(NH <sub>4</sub> )NO <sub>3</sub>	1.60	mg
Na <sub>2</sub> HPO <sub>4</sub>	8.00	mg
Distilled water	1000.00	ml

Final pH should be 7.6 ± 0.2 at 25°C. If using the complete medium from Difco add 37.40 g to 1 litre water.