760. MARINE THERMOCOCCUS MEDIUM

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 (N_2 for DSM 2476, DSM 5473, DSM 5474, DSM 9503, DSM 10343, DSM 10394, DSM 10395, DSM 11113, DSM 11836, DSM 12593, DSM 12819, DSM 12820, DSM 12597, DSM 12767, DSM 14981, DSM 15226, DSM 16538, DSM 17994, DSM 17882, DSM 22688, DSM 27149

or
$N_2:CO_2$ 80:20 for DSM 5262).

The final pH of the medium should be pH 5.8 for DSM 2476,
pH 6.0 for DSM 12767,
pH 6.5 for DSM 5262, DSM 10394, DSM 10395, DSM 12593 and DSM 16538,
pH 7.0 for DSM 11836, DSM 14981, DSM 15226, DSM 17882 DSM 17994 DSM 22688,
          DSM 27149, DSM 27260 DSM 27261
pH 7.2 for DSM 9503, DSM 10343, DSM 11113, and DSM 12819, and
pH 7.5 for DSM 5473, DSM 5474, DSM 12820, DSM 12597,
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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.