## ATCC medium: 2104 Marine Thermococcus medium

Marine Broth 2216 (BD 279110).....37.4 g Distilled water..............1.0 L

Dissolve the broth powder well. Filter through normal filter paper. An iron sediment will collect on the filter paper. Boil the medium in a water bath and cool under oxygen-free  $N_2$  gas. Adjust pH to 7.2. Reduce the medium by adding 5.0 ml of a neutralized  $Na_2S$  solution (0.5 g  $Na_2S$  in 5.0 ml water, neutralized to pH 7.0 with  $H_2SO_4$ ) for a final concentration of 0.05%. Dispense the medium under gas into aluminum seal tubes containing finely divided sulfur (0.5% w/v) and seal the tubes. Sterilize the medium for 3 consecutive days at 100C for 3 hours. The medium should not give a heavy black precipitate. If it does, the iron sediment was not adequately removed by filtering and the medium should be made again.