

ATCC Medium 2104: Marine Thermococcus Medium

Base Medium

Bacto Marine Broth (Difco 2216) 37.4 g
Distilled water 1000.0 mL

*Dissolve well and filter through normal filter paper. **Note:** An iron sediment will collect on the filter paper.*

Boil filtered medium in a water bath and cool under oxygen-free N₂ gas.

Adjust pH to 7.2.

Reduce the medium by adding 5 mL of a neutralized Na₂S solution (see below) for a final concentration of 0.05%.

Place 0.5% finely divided sulfur (see below) into aluminum/Hungate tubes and/or serum vials.

Dispense complete medium under oxygen-free N₂ gas and seal the tubes/serum vials. Sterilize for 3 consecutive days at 100°C for 3 hours.

Neutralized Na₂S Solution

Na₂S 0.5 g
DI Water 5.0 mL

*Adjust to **pH 7.0** using H₂SO₄.*

Sulfur (0.5% w/v)

Sulfur 0.5 g
Base medium + Na₂S solution (see above) 100.0 mL

Adjust as needed based on the volume of media being dispensed.

Note: *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.*

To make medium from scratch use the formulation below:

Difco Marine Broth

Peptone	5.0 g
Yeast Extract	1.0 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride	8.8 g
Sodium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide	0.08 g
Strontium Chloride	0.034 g
Boric Acid	0.022 g
Sodium Silicate	0.004 g
Sodium Fluoride	0.0024 g
Ammonium Nitrate	0.0016 g
Disodium Phosphate	0.008 g
Distilled water	1000.0 mL