

Medium HH

(1 liter)

Step I

600 ml deionized H₂O
10 mg EDTA
0.5 g KH₂PO₄
200 mg MgSO₄ · 7 H₂O
75 mg CaCl₂ · 2 H₂O
0.5 g NH₄Cl
1 g sodium acetate
0.1 g yeast extract
20 µg Vitamin B₁₂
1 ml trace elements¹
1 g Na pyruvate
1.63 g (10mM) bicine (Sigma)

Autoclave liquid mixture (25 minutes) in dispensing jar containing a dispensing bell connected to the jar by sterile rubber tubing.

Step II

2.5 g NaHCO₃
2.5 g Na₂CO₃
300 ml deionized H₂O

Autoclave carbonate/bicarbonate as a dry powder in a 500-ml bottle. After cooling and when you ready to assemble the medium, dissolve the powders in the 300 ml of sterile water.

Step III

0.6 g Na₂S · 9 H₂O
100 ml degassed (boiled) H₂O

Wash crystals of Na₂S · 9 H₂O in distilled water, dry on a towel, and weigh out. Dissolve the washed crystals in boiling d-H₂O and autoclave immediately.

Step IV

After cooling, add dissolved carbonate/bicarbonate (Step II) and sulfide (Step III) mixtures to the sterile bell jar medium (Step I), adjust the pH to 9.0 and immediately dispense into 17-ml screw-cap tubes. Make sure the tubes are completely filled with medium (leaving as small an air bubble as possible) and tightly capped. The final medium may form a slight greyish-black precipitate. Let the medium “age” for at least two days before using.

¹ Trace elements (per liter of distilled water):

EDTA	5.2 g	Na ₂ MoO ₄ · 2H ₂ O	188 mg
CoCl ₂ · 6 H ₂ O	190 mg	NiCl ₂ · 6H ₂ O	25 mg
MnCl ₂ · 4H ₂ O	100 mg	ZnCl ₂	70 mg
FeCl ₂ · 4H ₂ O	1.5 g	VoSO ₄ · 2H ₂ O	30 mg
H ₃ BO ₃	6 mg	Na ₂ WO ₄ · 2H ₂ O	2 mg
CuCl ₂ · 2H ₂ O	17 mg	NaHSeO ₃	2 mg

Note to users: Any trace elements solution will likely work