

**1312. FRESHWATER HD MEDIUM****Solution A:**

CaCO <sub>3</sub>	0.025	g
NaHCO <sub>3</sub>	0.025	g
Peptone	0.500	g
D-Glucose	0.100	g
Yeast extract	0.250	g
HEPES	2.380	g
Agar (Oxoid No. 1) if appropriate	14.000	g
Freshwater Solution	100.000	ml
Distilled water	900.000	ml

**Freshwater solution B:**

NH <sub>4</sub> Cl	5.3	mg
KH <sub>2</sub> PO <sub>4</sub>	14.0	mg
KNO <sub>3</sub>	100.0	mg
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	493.0	mg
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	147.0	mg
Distilled water	1000.0	ml

Dissolve carbonates in 900 ml distilled water by stirring for 5 h or longer. Add other components while stirring, adjust pH to 7.2 with KOH. Autoclave.

Autoclave solution A and B separately and combine after cooling.