

**854. HIPPEA MEDIUM**

NH <sub>4</sub> Cl	0.33	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.33	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.33	g
KCl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
NaCl	25.00	g
MOPS buffer (SIGMA)	3.00	g
Trace element solution (see medium 141)	10.00	ml
Yeast extract	0.10	g
Na-acetate	5.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Vitamin solution (see medium 141)	10.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, vitamins and sulfide), adjust pH to 5.5 - 6.0 and sparge the medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials which contain already an appropriate amount of sulfur powder. Autoclave 20 min at **110°C**! Prior to use add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins are sterilized by filtration. Adjust pH of complete medium to 6.0 - 6.2, if necessary.