

**1743. GHIORSEA MEDIUM**

NaCl	27.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	5.38	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	6.78	g
KCl	0.72	g
NH <sub>4</sub> Cl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.40	g
KH <sub>2</sub> PO <sub>4</sub>	0.05	g
Trace element solution (see medium 705)	10.00	ml
NaHCO <sub>3</sub>	1.70	g
Vitamin solution (see medium 141)	10.00	ml
Distilled water	980.00	ml

Dissolve ingredients (except vitamins), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to make it anoxic and until a pH of 6.5 is reached. Dispense medium under same gas atmosphere into anoxic serum vials to 20% of their volume and autoclave. After autoclaving add vitamins from a sterile stock solution prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Prior to inoculation add 5% (v/v) sterile H<sub>2</sub> and 5% (v/v) sterile air to the headspace by using syringes. Check pH of the complete medium and adjust pH to 6.5, if necessary. Incubate without shaking.

*Note: After growth has started cell density of the culture can be increased by replenishing H<sub>2</sub> and air every two days.*