

1743: GHIORSEA MEDIUM

NaCl	27.50	g
MgCl ₂ x 6 H ₂ O	5.38	g
MgSO ₄ x 7 H ₂ O	6.78	g
KCl	0.72	g
NH ₄ Cl	0.50	g
CaCl ₂ x 2 H ₂ O	1.40	g
KH ₂ PO ₄	0.05	g
Trace element solution	10.00	ml
NaHCO ₃	1.70	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except vitamins), then sparge medium with 80% N₂ and 20% CO₂ 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% N₂ gas atmosphere and sterilized by filtration. Prior to inoculation add 5% (v/v) sterile H₂ 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.

2. Note: After growth has started cell density of the culture can be increased by replenishing H₂ and air every two days.

Trace element solution (from medium 705)

Na ₂ -EDTA x 2 H ₂ O	0.50	g
CoCl ₂ x 6 H ₂ O	0.15	g
MnCl ₂ x 4 H ₂ O	0.10	g
FeSO ₄ x 7 H ₂ O	0.10	g
ZnCl ₂	0.10	g
AlCl ₃ x 6 H ₂ O	40.00	mg
Na ₂ WO ₄ x 2 H ₂ O	40.00	mg
Na ₂ SeO ₃ x 5 H ₂ O	30.00	mg
NiCl ₂ x 6 H ₂ O	20.00	mg
CuCl ₂ x 2 H ₂ O	20.00	mg
H ₃ BO ₃	10.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	10.00	mg
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7 using 2 N NaOH, then dissolve remaining compounds.



Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml