1011d. GALENEA MJ MEDIUM (CO2)

NaCl	30.00	g
K_2HPO4	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.18	g
KCl	0.33	g
NH_4Cl	0.25	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.01	g
Trace element solution (see medium 141)	10.00	ml
Na_2CO_3	2.50	g
$Na_2S_2O_3$ x 5 H_2O	1.50	g
Vitamin solution (see medium 141)	10.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, thiosulfate and vitamins), then sparge medium with 100% CO_2 gas for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add carbonate, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions. Solutions of vitamins and thiosulfate are sterilized by filtration and stored under 100% N_2 gas, whereas the stock solution of carbonate is prepared under 80% N_2 and 20% CO_2 gas mixture and autoclaved. Adjust pH of the complete medium to 6.7. After inoculation pressurize vessels to 0.5 bar overpressure with sterile 100% CO_2 gas and add sterile air in an amount that is equivalent to a volume of 20% of the headspace.