883: CALDIVIRGA MEDIUM

Trisodium citrate x 2 H_2O	2.94	g
$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Yeast extract (OXOID)	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur, yeast extract, vitamins and sulfide, then adjust the pH to 3.5 with 4 N H_2SO_4. Sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials which contain already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Autoclave at **105°C** for 20 min. Prior to inoculation add yeast extract, vitamins (sterilized by filtration) and sulfide from anoxic stock solutions prepared under 100% N_2 gas atmosphere. The final pH of the complete medium should be around 4.0.

2. After inoculation, pressurize the cultivation vessels to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Allen's trace element solution (from medium 88)

$MnCl_2$ x 4 H_2O	180.00	mg
$Na_2B_4O_7$ x 10 H_2O	450.00	mg
$ZnSO_4$ x 7 H_2O	22.00	mg
$CuCl_2$ x 2 H_2O	5.00	mg
Na_2MoO_4 x 2 H_2O	3.00	mg
$VOSO_4$ x 2 H_2O	3.00	mg
$CoSO_4$ x 7 H_2O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.

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