

1003. HYDROGEN-OXYDIZING MEDIUM

Place 1000 ml of anaerobic water (CO₂ water) in a flask. Add the following compounds:

MgSO ₄ x 7 H ₂ O	7.00	g
Na ₂ S ₂ O ₃	2.00	g
CaCl ₂ x 2 H ₂ O	0.40	g
KCl	0.48	g
MgCl ₂	0.78	g
MES	1.95	g

Add solutions A, B, and D (see recipes below):

Solution A	2.00	ml
Solution B	1.50	ml
Solution D (Trace minerals)	10.00	ml

pH the media to 6.0 and gas the flask containing the media with CO₂ for 20 min at least. Gas first culture tube in rack and place 5 ml of media in previous tube. Place stopper on loaded culture tube, crimp tube cap onto stopper. Autoclave the medium for 20 min., 121°C. Add 1 ml of O₂ to each tube before inoculation. After the inoculation the tubes are pressurized with H₂ (138 KP).

Solution A (100x solution):

NH ₄ Cl	100.00	g
MgCl ₂ x H ₂ O	100.00	g
CaCl ₂ x 2 H ₂ O	40.00	g
Distilled water	1000.00	ml

Adjust the pH to 4 with HCl.

Solution B (500x solution):

K ₂ HPO ₄ x 3 H ₂ O	200.00	g
Distilled water	1000.00	ml

Trace mineral solution (100x): *see next page*

Trace mineral solution (100x):

Na-EDTA x 2 H ₂ O	500.00	mg
CoCl ₂ x 6 H ₂ O	150.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
FeSO ₄ x 7 H ₂ O	100.00	mg
ZnCl ₂	100.00	mg
AlCl ₃ x 6 H ₂ O	40.00	mg
Na-tungstate x 2 H ₂ O	30.00	mg
CuCl	20.00	mg
Ni ₂ SO ₄ x 6 H ₂ O	20.00	mg
Se-acid	10.00	mg
HBO ₃	10.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	10.00	mg
Distilled water	1000.00	ml

Adjust the pH of the solution to 3.0 with HCl.