

540. ACETITOMACULUM MEDIUM

KH ₂ PO ₄	0.20	g
Na ₂ SO ₄	0.10	g
NH ₄ Cl	0.30	g
KCl	0.50	g
NaCl	7.00	g
MgCl ₂ x 6 H ₂ O	1.20	g
CaCl ₂ x 2 H ₂ O	0.15	g
Yeast extract	1.50	g
Trace element solution (see medium 141)	10.00	ml
NiCl ₂ x 6 H ₂ O solution (0.1% w/v)	1.00	ml
Na ₂ WO ₄ x 2 H ₂ O solution (0.1% w/v)	0.10	ml
Vitamin solution (see medium 141)	10.00	ml
Ascorbic acid	50.00	µg
Myo-inositol	50.00	µg
Niacinamide	50.00	µg
Choline chloride	50.00	µg
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
D-Glucose	5.00	g
L-Cysteine-HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, glucose and reducing agents), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add solid bicarbonate and equilibrate pH to 6.8 - 7.0 with 80% N₂ and 20% CO₂ gas mixture, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere.