

Solution A

KH ₂ PO ₄	0.30	g
K ₂ HPO ₄	0.30	g
NH ₄ Cl	1.00	g
Na ₂ SO ₄	3.00	g
NaCl	23.00	g
KCl	0.10	g
CaCl ₂	0.10	g
Yeast extract (BD-Difco)	0.10	g
Trace element solution	1.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Resazurin	1.00	mg
Distilled water	900.00	ml

1. To complete the medium, add the following stock solutions [Filter-sterilize NaHCO₃ solution and store under a N₂-CO₂ (4:1, v/v) gas mixture. The other solutions are autoclaved and stored anaerobically under a N₂ atmosphere] to the medium (per 4.5 ml):

Sodium lactate (1 M)	0.10	ml
MgCl ₂ x 6 H ₂ O (15%)	0.10	ml
NaHCO ₃ (5%)	0.20	ml
Na ₂ S x 9 H ₂ O (2%)	0.10	ml

2. Mix ingredients and adjust pH to 7.2. Distribute 0.9 volume of the medium into tubes (e.g., 4.5 ml of the medium in the Hungate tubes) under a N₂-CO₂ (4:1, v/v) gas mixture, seal with butyl rubber stoppers and autoclave at 110C for 45 min.