

Main sol. J197

KH ₂ PO ₄	0.75	g
K ₂ HPO ₄	0.75	g
MgCl ₂ x 6 H ₂ O	0.36	g
NH ₄ Cl	1.00	g
Yeast extract (BD-Difco)	0.10	g
Polypeptone (Nihon Pharm. Co.)	0.10	g
Sodium acetate	2.50	g
Trimethylamine-HCl	8.00	g
Trace mineral solution	9.00	ml
Trace vitamins	10.00	ml
NaHCO ₃	0.80	g
Na ₂ S x 9 H ₂ O	0.50	g
L-Cysteine HCl x H ₂ O	0.50	g
Resazurin	1.00	mg
Distilled water	981.00	ml

Mix ingredients except NaHCO₃, Trace vitamins, Na₂S x 9H₂O and cysteine x HCl x H₂O, and autoclave under a N₂-CO₂ (80:20) atmosphere. Filter-sterilize NaHCO₃ as an 8% solution and Trace vitamins while gassing with N₂. Separately autoclave Na₂S x 9H₂O and cysteine x HCl x H₂O as 5% solutions under a N₂ atmosphere. Aseptically and anaerobically add NaHCO₃ and Trace vitamins to the medium under a N₂-CO₂ (80:20) atmosphere. Prior to inoculation, add the sterile Na₂S x 9H₂O and cysteine x HCl x H₂O solutions, and adjust pH to 6.8.