

Main sol. J843

KH ₂ PO ₄	0.30	g
K ₂ HPO ₄	0.30	g
NH ₄ Cl	1.00	g
NaCl	20.00	g
KCl	0.10	g
CaCl ₂ x 2 H ₂ O	0.10	g
Trace element solution	1.00	ml
Yeast extract	2.00	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	4.96	g
L-Cysteine HCl x H ₂ O	0.50	g
Resazurin	1.00	mg
Distilled water	925.00	ml

1. Mix components thoroughly and autoclave under a N₂-CO₂ (4:1, v/v) gas mixture. After cooling, add the following solutions from anaerobic stocks (autoclaved or *filter-sterilized):

NaHCO ₃ (*, 8%)	25.00	ml
MgCl ₂ x 6 H ₂ O (10%)	30.00	ml
Sodium acetate (1.0 M)	20.00	ml

2. Then, aseptically distribute the medium into culture vessels under a stream of H₂-CO₂ (4:1, v/v) and seal with butyl rubber stoppers. Prior to inoculation, add per liter the following solution from an anaerobic stock (autoclave and store under a N₂ atmosphere):

Na ₂ S x 9 H ₂ O (5%)	8.00	ml
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3. Pressurize inoculated culture vessels to 200 kPa H₂-CO₂ (4:1, v/v).