



Main sol. J981

KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.45	g
NaCl	1.70	g
KCl	0.50	g
MgCl ₂ x 6 H ₂ O	0.40	g
CaCl ₂ x 2 H ₂ O	0.16	g
Na ₂ SO ₄	4.00	g
FeCl₂ solution	1.00	ml
Trace element solution	1.00	ml
Selenite-tungstate solution	1.00	ml
Resazurin	1.00	mg
Distilled water	1000.00	ml

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

NaHCO ₃ (8.0%)	30.00	ml
Vitamin solution	1.00	ml
Thiamine solution	1.00	ml
Vitamin B₁₂ solution 0.05 g/L	1.00	ml

2. Distribute the medium into sterile culture vessels (e.g., 50 ml per 120 ml bottle) under a N₂-CO₂ (4:1, v/v) gas mixture, and seal with sterile butyl rubber stoppers. Prior to inoculation, add per liter the following solutions (autoclaved or *filter-sterilized and stored under a N₂ atmosphere) in the order listed:

Phenol (freshly prepared, *, 0.1 M)	10.00	ml
Na ₂ S x 9 H ₂ O (5%)	10.00	ml