

Main sol. 319

Casamino acids (BD Bacto)	2.00	g
Nutrient broth (BD Difco)	2.00	g
Yeast extract	2.00	g
L-Glutamic acid	4.00	g
NaCl	105.00	g
KCl	0.75	g
Modified Wolin's mineral solution	10.00	ml
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
MgCl ₂ x 6 H ₂ O	10.00	g
CaCl ₂ x 2 H ₂ O	3.70	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except cysteine, magnesium chloride, calcium chloride and vitamins), bring medium to the boil, then cool to room temperature under 100% N₂ gas atmosphere. Thereafter, add and dissolve cysteine, adjust pH to 6.5 and dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add magnesium chloride and calcium chloride from sterile anoxic stock solutions autoclaved under 100% N₂ gas and vitamins from a filter-sterilized stock solution prepared under N₂ gas.

2. Note: For agar medium add 20.00 g/l agar, 5.00 g/l CaCO₃, and 2.00 g/l soluble starch.