

## Main sol. 806

KCl	0.33	g
MgCl <sub>2</sub> x 2 H <sub>2</sub> O	2.70	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.40	g
NH <sub>4</sub> Cl	0.25	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
Na <sub>2</sub> SeO <sub>3</sub>	1.00	µg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	1.00	µg
NaHCO <sub>3</sub>	1.00	g
NaCl	18.00	g
Resazurin	1.00	mg
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Yeast extract	1.00	g
Trypticase	1.00	g
Sulphur	5.00	g
<b>Modified Wolin's mineral solution II</b>	10.00	ml
<b>Wolin's vitamin solution</b>	10.00	ml
Distilled water	980.00	ml

Prepare the medium without NaHCO<sub>3</sub>, cysteine hydrochloride, Na<sub>2</sub>S x 9 H<sub>2</sub>O, and vitamin solution. Boil the medium and cool under N<sub>2</sub>. Add the NaHCO<sub>3</sub> to the cooled medium and adjust the pH to about pH 7.2. Sterilise the medium at 100°C for 3 hours on 3 consecutive days. Reduce the medium by adding 10% neutralised sodium sulphide and cysteine hydrochloride from sterile stock solutions. The vitamins are added from a sterile (filter sterilised) stock solution prepared under nitrogen. The final pH is 7.2.