

**Main sol. 558**

$\text{KH}_2\text{PO}_4$	1.50	g
$\text{Na}_2\text{HPO}_4 \times 12 \text{ H}_2\text{O}$	4.20	g
$\text{NH}_4\text{Cl}$	0.50	g
$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$	0.36	g
$\text{Na}_2\text{SO}_4$	1.00	g

**Modified Wolin's mineral solution**

Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	10.00	g
Yeast extract	2.00	g

**Wolin's vitamin solution (10x)**

Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl $\times \text{H}_2\text{O}$	0.50	g
$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, yeast extract, vitamin solution and reducing agents), adjust pH to 3.5 using HCl and sparge medium with 80%  $\text{N}_2$  and 20%  $\text{CO}_2$  gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials. After autoclaving add glucose, yeast extract, vitamin solution, cysteine and sulfide from sterile anoxic stock solutions prepared under 100%  $\text{N}_2$  gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 3.5, if necessary.