

## Main sol. 876

Na <sub>2</sub> SO <sub>4</sub>	4.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.25	g
NaCl	20.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	9.80	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Na-caproate	0.50	g
Na-butyrate	0.30	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
<b>Seven vitamins solution</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	1000.00	ml

1. Dissolve all ingredients except carbonate, caproate, butyrate, vitamins and sulfide, then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add caproate, butyrate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.9, if necessary.

2. Note: Growth can be stimulated by supplementing the medium with 1% (v/v) of fatty acid mixture (see medium 119).