

## Main sol. 666

<b>Clarified rumen fluid</b>	400.00	ml
K <sub>2</sub> HPO <sub>4</sub>	0.23	g
KH <sub>2</sub> PO <sub>4</sub>	0.23	g
NaCl	0.45	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.45	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.06	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.09	g
Indigocarmine	5.00	mg
NaHCO <sub>3</sub>	6.40	g
Disodium succinate	5.00	g
Yeast extract	5.00	g
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	600.00	ml

1. Dissolve ingredients (except bicarbonate, succinate, yeast extract, cysteine and sulfide), bring medium to the boil, then cool to room temperature under 100% CO<sub>2</sub> gas atmosphere. Add the bicarbonate and equilibrate the medium with the CO<sub>2</sub> gas to pH 6.8. Distribute under 100% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Thereafter, add succinate, yeast extract, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere. Adjust pH of complete medium to 6.7 - 6.8, if necessary.

2. Note: Supplementing the medium with 1.50 g/l agar stimulates growth of strains after resuscitation from ampoules.