

## Main sol. J146

Polypeptone (BD-BBL)	5.00	g
Heart Infusion Broth (BD-Difco)	5.00	g
Yeast extract	5.00	g
Pectin	0.80	g
Glucose	0.80	g
Starch	0.80	g
Sucrose	0.80	g
Maltose	0.80	g
Sodium pyruvate	0.80	g
Ribose	0.80	g
K <sub>2</sub> HPO <sub>4</sub>	2.00	g
NaCl	5.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
L-Cysteine HCl x H <sub>2</sub> O	0.68	g
Clarified rumen fluid	500.00	ml
Distilled water	500.00	ml
Agar	1.60	g

Adjust pH to 7.0. Prepare medium anaerobically and dispense into rubber-stoppered tubes under oxygen-free N<sub>2</sub>. Add inactivated sterile rabbit serum to give final concentration in each tube of 5%. Add filter-sterilized thiamine pyrophosphate to each tube to give a final concentration of 0.75 mg/100 ml. Subculture with a Pasteur pipette (20-30 drops). Gas phase for culture system should be oxygen-free N<sub>2</sub> or N<sub>2</sub>-CO<sub>2</sub> (90:10).