



Main sol. J144

Combine the above ingredients and bring to a boil in a round bottom flask while flushing with O₂-free N₂-H₂-CO₂ (85:10:5) gas mixture. Stopper anaerobically, wire or seal closed and autoclave at 121C for 20 min. After autoclaving, allow to cool, open without admitting oxygen and while flushing with gas, add the following ingredients which have been filter-sterilized ahead of time:

Heart Infusion Broth (BD-Difco)	1.25	g
Trypticase peptone (BD-BBL)	1.00	g
Yeast extract (BD-BBL)	0.25	g
Sodium thioglycolate	0.05	g
L-Cysteine HCl x H ₂ O	0.10	g
L-Asparagine	0.25	g
Glucose	0.20	g
Resazurin (0.1%)	0.10	ml
Distilled water	94.00	ml
NaHCO ₃ (7.5%)	2.67	ml
TPP/VFA mixture	2.00	ml
Rabbit serum (heat-inactivated at 56C for 1 hr)	2.00	ml