

Main sol. 320

K ₂ HPO ₄ x 3 H ₂ O	1.00	g
NH ₄ Cl	1.00	g
KCl	0.50	g
MgSO ₄ x 7 H ₂ O	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Yeast extract	0.50	g
Clarified rumen fluid	20.00	ml
Sludge fluid , alternative (optional)	20.00	ml
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.15	g
Na ₂ CO ₃	1.00	g
Cellobiose	5.00	g
Cellulose, MN 301 (optional)	10.00	g
Na ₂ S x 9 H ₂ O	0.15	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except cysteine, carbonate, cellobiose and sulfide), bring medium to the boil, then cool to room temperature under 80% N₂ and 20% CO₂ gas mixture and add cysteine. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sulfide from a sterile anoxic stock solution prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Sterilize cellobiose separately by filtration under 100% N₂ gas. Adjust pH of the complete medium to 7.0, if necessary.

2. Note: Some strains can be adapted to cellulose as substrate using 10.0 g/l cellulose powder MN 301 (MACHEREY-NAGEL).