

Main sol. 520

(NH ₄) ₂ SO ₄	1.30	g
KH ₂ PO ₄	1.50	g
K ₂ HPO ₄ x 3 H ₂ O	2.90	g
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	1.25	ml
Trace element solution SL-10	1.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	75.00	mg
Cellobiose	6.00	g
Cellulose, MN 301 (optional)	10.00	g
Na ₂ CO ₃	1.50	g
L-Cysteine HCl x H ₂ O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except magnesium chloride, calcium chloride, cellobiose, cysteine and carbonate, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add magnesium chloride, calcium chloride and cellobiose from anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂. Cellobiose has to be sterilized by filtration. Prior to inoculation add cysteine from a sterile anoxic stock solution prepared under 100% N₂ gas and adjust pH to 7.2.

2. Note: Some strains can be adapted to cellulose as substrate using 10.00 g/l cellulose powder MN 301 (MACHEREY-NAGEL).