

122. HUNGATEICLOSTRIDIUM MEDIUM

(NH ₄) ₂ SO ₄	1.30	g
MgCl ₂ x 6 H ₂ O	2.60	g
KH ₂ PO ₄	1.43	g
K ₂ HPO ₄	5.50	g
CaCl ₂ x 2 H ₂ O	0.13	g
Na ₂ -β-glycerol phosphate x 4 H ₂ O	6.00	g
FeSO ₄ x 7 H ₂ O solution (0.1% w/v in 0.1 N H ₂ SO ₄)	1.10	ml
L-Glutathione reduced	0.25	g
Yeast extract	4.50	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Cellobiose	5.00	g
Distilled water	1000.00	ml

Dissolve ingredients except cellobiose, sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Then adjust pH to 7.0 - 7.2, distribute under same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave.

Cellobiose is added to the sterile medium from an anoxic 10% (w/v) stock solution prepared under 100% N₂ gas and sterilized by filtration. Some strains can be adapted to cellulose as substrate using 10.00 g/l cellulose (Avicel or MN 301, MACHEREY-NAGEL).

Note: A white precipitate forms after mixing the ingredients of this medium, but this has no negative effect on growth.