

104c: PY + X MEDIUM (N₂/CO₂)

This recipe contains strain-specific modifications for *Clostridium leptum* DSM 753 *

Trypticase peptone (BD BBL)	5.00	g
Meat peptone (pepsin-digested)	5.00	g
Yeast extract	10.00	g
Salt solution	40.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ CO ₃	1.00	g
D-Glucose	5.00	g
D-Maltose	10.00	g
Distilled water	960.00	ml

Dissolve ingredients (except cysteine, carbonate and glucose) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add cysteine, then dispense under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose from a sterile anoxic stock solution prepared under 100% N₂ gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Adjust pH of complete medium to 7.0, if necessary.

* Replace glucose with 10.0 g/l D-maltose added after autoclaving from an anoxic stock solution sterilized by filtration.

Salt solution (from medium 104)

CaCl ₂ x 2 H ₂ O	0.25	g
MgSO ₄ x 7 H ₂ O	0.50	g
K ₂ HPO ₄	1.00	g
KH ₂ PO ₄	1.00	g
NaHCO ₃	10.00	g
NaCl	2.00	g
Distilled water	1000.00	ml