

## 104c: PY + X MEDIUM (N<sub>2</sub>/CO<sub>2</sub>)

This recipe contains strain-specific modifications for *Clostridium ragsdalei* DSM 15248 \*

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

Dissolve ingredients (except cysteine, carbonate and glucose) and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Add cysteine, then dispense under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Adjust pH of complete medium to 7.0, if necessary.

\* Adjust pH of medium to 6.0.

### Salt solution (from medium 104)

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