

104: PYG MEDIUM (modified)

This recipe contains strain-specific modifications for *Fretibacterium fastidiosum* DSM 25557 *

Final pH: 7.2

Final volume: 1000 ml

Trypticase peptone	5.00	g
Peptone	5.00	g
Yeast extract	10.00	g
Beef extract	5.00	g
Glucose	5.00	g
K ₂ HPO ₄	2.00	g
Tween 80	1.00	ml
Salt solution	40.00	ml
Resazurin	1.00	mg
Vitamin K₁ solution	0.20	ml
Haemin solution	10.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Clarified rumen fluid	50.00	ml/l
Dithiothreitol	0.20	g/l
Horse serum	50.00	ml/l
Distilled water	950.00	ml

The vitamin K₁, haemin solution and the cysteine are added after the medium has been boiled and cooled under CO₂. Adjust pH to 7.2 using 8 N NaOH. Distribute under N₂ and autoclave.

* Supplement medium after autoclaving with 50.0 ml/l clarified rumen fluid (see medium 1310), 50.0 ml/l horse serum (Bio & Sell PFD.SE.0500) and 0.2 g/l dithiothreitol (DTT) added from filter-sterilized anoxic stock solutions.

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

Haemin solution (from medium 78)

Haemin	50.00	mg
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NaOH (1 N)	1.00	ml
Distilled water	100.00	ml

Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.

Vitamin K₁ solution (from medium 78)

Vitamin K ₁	0.10	ml
Ethanol (95 %)	20.00	ml

Dissolve 0.1 ml of vitamin K₁ in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.