

104. PYG MEDIUM (modified)

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 (see below)	40.00	ml
Resazurin	1.00	mg
Vitamin K ₁ solution (see below)	0.20	ml
Haemin solution (see below)	10.00	ml
Cysteine-HCl x H ₂ O	0.50	g
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.

Salt solution:

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:

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

Vitamin K₁ solution:

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

For [DSM 23669](#) and [DSM 25557](#) 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.