

## 104: PYG MEDIUM (modified)

This recipe contains strain-specific modifications for *Holdemania massiliensis* DSM 26143 \*

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 <sub>2</sub> HPO <sub>4</sub>	2.00	g
Tween 80	1.00	ml
<b>Salt solution</b>	40.00	ml
Resazurin	1.00	mg
<b>Vitamin K<sub>1</sub> solution</b>	0.20	ml
<b>Haemin solution</b>	10.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
<b>Haemin</b>	5.00	mg/l
Distilled water	950.00	ml

The vitamin K<sub>1</sub>, haemin solution and the cysteine are added after the medium has been boiled and cooled under CO<sub>2</sub>. Adjust pH to 7.2 using 8 N NaOH. Distribute under N<sub>2</sub> and autoclave.

\* Plus haemine (5µg/ml) plus Vit K<sub>1</sub>; strictly anaerobic

### 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

### Haemin solution (from medium 78)

Haemin	50.00	mg
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

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sterilize. Store refrigerated.

### **Vitamin K<sub>1</sub> solution** (from medium 78)

Vitamin K <sub>1</sub>	0.10	ml
Ethanol (95 %)	20.00	ml

Dissolve 0.1 ml of vitamin K<sub>1</sub> in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.