

**1455: ALTERNATIVE RUMEN BACTERIA MEDIUM**

K <sub>2</sub> HPO <sub>4</sub>	0.23	g
KH <sub>2</sub> PO <sub>4</sub>	0.23	g
NaCl	0.45	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.45	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.06	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.09	g
Brain heart infusion (BD BBL)	6.00	g
Yeast extract	5.00	g
<b>Volatile fatty acid mixture</b>	3.10	ml
<b>Haemin solution (0.05% w/v)</b>	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	6.40	g
Disodium succinate	5.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, succinate, vitamins, cysteine and sulfide, then sparge medium with 100% CO<sub>2</sub> gas for 30 - 45 min to make it anoxic. Add the bicarbonate and equilibrate the medium with the CO<sub>2</sub> gas to reach a pH between 6.5 and 7.0. Dispense medium under 100% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add succinate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. The vitamin solution should be sterilized by filtration. Prior to use adjust pH of complete medium to 6.6 - 6.8, if necessary.

**Volatile fatty acid mixture** (from medium 330)

Acetic acid	548.50	ml
Propionic acid	193.50	ml
Butyric acid	129.00	ml
n-Valeric acid	32.25	ml
iso-Butyric acid	32.25	ml
DL-2-Methylbutyric acid	32.25	ml
iso-Valeric acid	32.25	ml

**Haemin solution** (from medium 78)

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

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

### Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml