336: OXOBACTER PFENNIGII MEDIUM

Solution A	972.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	3.00	ml

Sparge solution A with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, distribute under same gas atmosphere 8.9 ml medium per Hungate-type tube and autoclave. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solution C is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Autoclave solutions D and E under 100% N_2 gas. To complete the medium add appropriate amounts of the solutions B to E to the sterile solution A. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

Solution A

Mineral solution	50.00	ml
Trace element solution SL-10	1.00	ml
Yeast extract	2.00	g
Na-vanillate	2.00	g
Clarified rumen fluid	300.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	620.00	ml

Adjust pH to 6.9 before autoclaving.

Solution B

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution C

Seven vitamins solution	1.00	ml

Solution D

L-Cysteine HCl x H_2O	0.30	g
Distilled water	10.00	ml

Solution E

Na_2S x 9 H_2O	90.00	mg

Distilled water	3.00	ml

Mineral solution (from medium 335)

KH_2PO_4	10.00	g
$MgCl_2$ x 6 H_2O	6.60	g
NaCl	8.00	g
NH_4Cl	8.00	g
$CaCl_2$ x 2 H_2O	1.00	g
Distilled water	1000.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml