213: SYNTHROPHOMONAS MEDIUM (SULFATE FREE)

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

1. Add and dissolve ingredients of solution A, adjust pH to 7.2 and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Solution B is prepared under 80% N_2 and 20% CO_2 gas atmosphere and autoclaved. Solution C is prepared under 100% N_2 gas and sterilized by filtration. Solutions D, E and F are autoclaved under 100% N_2 gas atmosphere. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in in the sequence as indicated.

2. Note: Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days . After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.

For DSM 2909, DSM 2984: Replace butyric acid with 1.50 g/l acetoin.

For DSM 15682, DSM 16215: Replace butyric acid with 1.70 g/l crotonic acid.

For DSM 102353: Replace butyric acid with 2.00 g/l Na-benzoate.

Solution A

KH_2PO_4	0.50	g
$MgCl_2$ x 6 H_2O	0.33	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml
Clarified rumen fluid	50.00	ml
Trypticase peptone (BD BBL)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	900.00	ml

Solution B

Na_2CO_3	1.50	g

Distilled water	30.00	ml

Solution C

Seven vitamins solution	1.00	ml

Solution D

Butyric acid	1.70	g
Distilled water	10.00	ml

Solution E

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

Solution F

Na_2S x 9 H_2O	0.30	g
Distilled water	10.00	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