213. SYNTHROPHOMONAS MEDIUM (SULFATE FREE)

Solution A:		
Mineral solution (see medium 212)	50.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Rumen fluid, clarified (see medium 1310)	50.00	ml
Trypticase peptone (BD BBL)	1.00	g
Butyric acid	1.70	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	850.00	ml
Solution B:		
Na_2CO_3	1.50	g
Distilled water	30.00	ml
Solution C:		
Vitamin solution (see medium 503)	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	0.30	g
Distilled water	10.00	ml

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* and *E* are autoclaved under 100% N_2 gas atmosphere. To complete the medium appropriate amounts of s*olutions B* to *E* are added to the sterile *solution A* in in the sequence as indicated.
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.

Continued next page

For DSM 2909, DSM 2984 and DSM 21899 replace butyric acid with 1.50 g/l acetoin.

For DSM 15682 and 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.