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	830.00	ml
Solution B:		
$NaHCO_3$	3.50	g
Distilled water	50.00	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.

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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.