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:
- Na₂CO₃ 1.50 g
- Distilled water 30.00 ml

Solution C:
- Vitamin solution (see medium 503) 1.00 ml

Solution D:
- L-Cysteine-HCl x H₂O 0.30 g
- Distilled water 10.00 ml

Solution E:
- Na₂S x 9 H₂O 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% N₂ and 20% CO₂ 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% N₂ and 20% CO₂ gas atmosphere and autoclaved. Solution C is prepared under 100% N₂ gas and sterilized by filtration. Solutions D and E are autoclaved under 100% N₂ gas atmosphere. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A 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.