

212. SYNTHROPHOMONAS MEDIUM

Solution A:

Mineral solution (see below)	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 ₂ SO ₄	2.80	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
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Solution D:

L-Cysteine-HCl x H ₂ O	0.30	g
Distilled water	10.00	ml

Solution E:

Na ₂ S x 9 H ₂ O	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 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

Mineral solution:

KH ₂ PO ₄	10.00	g
MgCl ₂ x 6 H ₂ O	6.60	g
NaCl	8.00	g
NH ₄ Cl	8.00	g
CaCl ₂ x 2 H ₂ O	1.00	g
Distilled water	1000.00	ml

For DSM 2805 replace butyric acid with 1.50 g/l Na-propionate and supplement medium with 1.00 g/l D-glucose added from a sterile anoxic stock solution sterilized by filtration.

For DSM 16706 replace butyric acid with 1.50 g/l Na-propionate.

For DSM 26217 omit rumen fluid and butyric acid from *solution A* and add to the complete medium 7.00 g/l sucrose from a sterile anoxic stock solution.

For DSM 102352 replace butyric acid with 2.00 g/l Na-benzoate.