

506. METHANOBACTERIUM ESPANOLAE MEDIUM

Na-acetate x 3 H ₂ O	2.50	g
(NH ₄) ₂ SO ₄	0.45	g
K ₂ HPO ₄	0.29	g
KH ₂ PO ₄	0.18	g
MgSO ₄ x 7 H ₂ O	0.12	g
CaCl ₂ x 2 H ₂ O	0.06	g
NaCl	0.05	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	0.50	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.25	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve carbonate, then dispense medium into anoxic Hungate-type tubes or serum vials under 80% H₂ and 20% CO₂ gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use check pH of complete medium and adjust to 5.8 - 6.0, if necessary.

Note: After growth has started and the culture is becoming turbid add sterile 80% H₂ and 20% CO₂ gas mixture to 0.5 - 1 bar overpressure.

For [DSM 19849](#) adjust pH of complete medium to 7.0 – 7.2 with a sterile anoxic stock solution of Na₂CO₃ prepared under 80% N₂ and 20% CO₂ gas mixture. After inoculation tubes are pressurized with sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure.