

814. METHANOBACTERIUM SUBTERRANEUM MEDIUM

NH ₄ Cl	0.40	g
MgCl ₂ x 6 H ₂ O	0.03	g
NaCl	0.45	g
K ₂ HPO ₄	0.50	g
FeSO ₄ x 7 H ₂ O solution (0.1% w/v in 0.1 N H ₂ SO ₄)	3.00	ml
Trace element solution (see medium 141)	10.00	ml
Tryptone (BD Bacto)	1.00	g
Na-formate	3.20	g
Na-acetate	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	2.00	g
Vitamin solution (see medium 503)	1.00	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 bicarbonate, vitamins, cysteine and sulfide, then sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to use adjust pH of complete medium to 8.0 - 8.5.