

374. METHANOSPIRILLUM SK MEDIUM

KH ₂ PO ₄	0.50	g
MgSO ₄ x 7 H ₂ O	0.40	g
NaCl	0.40	g
NH ₄ Cl	0.40	g
CaCl ₂ x 2 H ₂ O	0.05	g
FeSO ₄ x 7 H ₂ O solution (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract (OXOID)	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
Sludge fluid (see medium 119)	50.00	ml
Fatty acid mixture (see medium 119)	20.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
Isopropyl alcohol	7.50	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	940.00	ml

Dissolve ingredients except bicarbonate, isopropyl alcohol and sulfide. Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add isopropyl alcohol and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use check pH of complete medium and adjust to 7.0 – 7.2, if necessary.