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% N$_2$ and 20% CO$_2$ 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% N$_2$ gas. Prior to use check pH of complete medium and adjust to 7.0 – 7.2, if necessary.