1051. TEPIDANAEROBACTER MEDIUM

KH$_2$PO$_4$ 0.14 g
MgCl$_2$ x 6 H$_2$O 0.20 g
CaCl$_2$ x 2 H$_2$O 0.15 g
NH$_4$Cl 0.54 g
Trace element solution (see medium 144) 1.00 ml
Na-resazurin solution (0.1% w/v) 0.50 ml
Na$_2$CO$_3$ 1.50 g
Yeast extract 2.30 g
Vitamin solution (see medium 141) 2.00 ml
D-Glucose 2.20 g
L-Cysteine-HCl x H$_2$O 0.30 g
Na$_2$S x 9 H$_2$O 0.30 g
Distilled water 1000.00 ml

Dissolve ingredients (except carbonate, yeast extract, vitamins, glucose, cysteine and sulfide), then sparge medium with 80% N$_2$ and 20% CO$_2$ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins (sterilized by filtration), glucose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N$_2$ gas and carbonate from a sterile anoxic stock solution prepared under 80% N$_2$ and 20% CO$_2$ gas mixture. Adjust pH of the complete medium to 7.0-7.2, if necessary.

For DSM 21860 omit glucose, increase amount of ammonium chloride to 2.50 g/l, decrease amount of yeast extract to 0.20 g/l and supplement medium with 1.00 ml/l selenite-tungstate solution (see medium 385). As substrate add 1.10 g/l betaine to the autoclaved medium from an anoxic stock solution sterilized by filtration.