

**716. AMMONIFEX DEGENSII MEDIUM**

$K_2HPO_4 \times 3 H_2O$	0.30	g
$KH_2PO_4$	0.22	g
$(NH_4)_2SO_4$	0.22	g
NaCl	1.00	g
$MgSO_4 \times 7 H_2O$	0.09	g
$CaCl_2 \times 2 H_2O$	0.06	g
$FeSO_4 \times 7 H_2O$ solution (0.1% w/v in 0.1 N $H_2SO_4$ )	2.00	ml
$NiCl_2 \times 6 H_2O$ solution (0.1% w/v)	0.20	ml
Trace element solution (see medium 141)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
$KNO_3$	1.00	g
$Na_2CO_3$	1.50	g
$Na_2S \times 9 H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except nitrate, carbonate and sulfide), then sparge medium with 80%  $H_2$  and 20%  $CO_2$  gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic serum vials (e.g., 10 ml medium in 25 ml Balch-type tubes) and autoclave. Prior to use add nitrate from a sterile anoxic stock solution 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. Before inoculation add sulfide from a sterile anoxic stock solution prepared under 100%  $N_2$  gas and adjust pH of the medium to 7.2. After inoculation pressurize vials to 2 bar overpressure with sterile 80%  $H_2$  and 20%  $CO_2$  gas mixture.