

716: AMMONIFEX DEGENSII MEDIUM

K ₂ HPO ₄ x 3 H ₂ O	0.30	g
KH ₂ PO ₄	0.22	g
(NH ₄) ₂ SO ₄	0.22	g
NaCl	1.00	g
MgSO ₄ x 7 H ₂ O	0.09	g
CaCl ₂ x 2 H ₂ O	0.06	g
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
NiCl ₂ x 6 H ₂ O (0.1% w/v)	0.20	ml
Modified Wolin's mineral solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
KNO ₃	1.00	g
Na ₂ CO ₃	1.50	g
DL-Dithiothreitol	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except nitrate, carbonate, and dithiothreitol), then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic serum vials (e.g., 10 ml medium in 25 ml Balch-type tubes) and autoclave. Before use, add nitrate from a sterile anoxic stock solution prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Before inoculation add dithiothreitol from an anoxic stock solution sterilized by filtration under 100% N₂ gas and adjust the pH of the medium to 7.2.

2. After growth has started pressurize vials to 0.5 bar overpressure with a sterile 80% H₂ and 20% CO₂ gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g



Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.