718: PETROTOGA MEDIUM

KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	18.00	g
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 7 H_2O (0.1% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	1.00	g
D-Glucose	5.00	g
Yeast extract	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, glucose, yeast extract, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate, adjust pH to 6.5 and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add glucose, yeast extract, vitamins, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins should be sterilized by filtration. Prior to inoculation adjust pH of complete medium to 6.5 - 7.7, if necessary.

For DSM 10691: Supplement medium with 1.00 g/l Trypticase peptone and increase amount of yeast extract to 1 g/l.

For DSM 13781: Supplement medium with 5.00 g/l $Na_2S_2O_3$ x 5 H_2O and 5.00 g/l Trypticase peptone added from anoxic stock solutions sterilized by filtration. Omit D-Glucose. Adjust amount of yeast extract to 2.00 g/l.

For DSM 13782: Supplement medium with 5.00 g/l $Na_2S_2O_3$ x 5 H_2O and 1.00 g/l Trypticase peptone added from anoxic stock solutions sterilized by filtration. Increase amount of yeast extract to 1.00 g/l.

For DSM 14811: Supplement medium with 2.00 g/l $Na_2S_2O_3$ x 5 H_2O added from a sterile anoxic stock solution sterilized by filtration.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g

NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	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.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml