960: PELOTOMACULUM MEDIUM

This recipe contains strain-specific modifications for *Oligosphaera ethanolica* DSM 24202 *

Final pH: 7.0
Final volume: 1002 ml

KH_2PO_4	0.14	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.54	g
Trace element solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
~~Na-pyruvate~~	~~2.20~~	~~g~~
~~Yeast extract (OXOID)~~	~~0.10~~	~~g~~
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
D-Glucose	0.18	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, pyruvate, yeast extract, vitamins, and reducing agents) and 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 pyruvate, yeast extract, vitamins, 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 atmosphere. Stock solutions of vitamins and pyruvate should be sterilized by filtration. The pH of the complete medium should be 7.0.

2. Note: Use 10% (v/v) as inoculum.

* Omit yeast extract and replace pyruvate with 0.18 g/l D-glucose added from an anoxic stock solution sterilized by filtration. Use 10% (v/v) as inoculum.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2$ x 4 H_2O	1.00	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g

Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.10	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Na_2WO_4 x 2 H_2O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

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