Microorganisms



960: PELOTOMACULUM MEDIUM

KH ₂ PO ₄	0.14	g
$MgCl_2 \times 6 H_2O$	0.20	g
CaCl ₂ x 2 H ₂ O	0.15	g
NH ₄ Cl	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 ₂ O	0.30	g
$Na_2S \times 9 H_2O$	0.30	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.

For <u>DSM 13752</u>, <u>DSM 21041</u>: Replace pyruvate with 0.92 ml/l ethanol.

For DSM 14795: Replace pyruvate with 0.86 g/l Na-crotonate.

For <u>DSM 14947</u>: Replace pyruvate with 0.72 g/l Na-benzoate.

For DSM 15200, DSM 15578, DSM 16145: Replace pyruvate with 2.00 g/l Na-propionate.

For <u>DSM 16121</u>: Replace pyruvate with 1.50 g/l Na-2,5-dihydroxybenzoate.

For <u>DSM 16494</u>, <u>DSM 22288</u>: Replace pyruvate with 2.72 g/l Na-formate and 0.10 g/l Na -acetate. Supplement medium with 0.08 g/l coenzyme M (2-mercaptoethanesulfonic acid).

For <u>DSM 17711</u>, <u>DSM 23604</u>: Replace pyruvate with 0.10 g/l Na-acetate and pressurize serum vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

For <u>DSM 17771</u>: Supplement medium with 1.42 g/l Na_2SO_4 and replace pyruvate with 0.41 g/l 4-hydroxybenzoic acid added from a sterile, neutralized and anoxic stock solution. Increase concentration of yeast extract to 0.20 g/l.

For <u>DSM 18709</u>, <u>DSM 26646</u>: Replace pyruvate with 0.86 g/l Na-crotonate and increase concentration of yeast extract to 0.50 g/l.

Microorganisms

960: PELOTOMACULUM MEDIUM



For <u>DSM 24196</u>: Increase amount of yeast extract to 0.20 g/l and replace pyruvate with 0.18 g/l D-glucose added from an anoxic stock solution sterilized by filtration. Use 5% (v/v) as inoculum.

For <u>DSM 24202</u>: 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.

For <u>DSM 102911</u>: Increase amount of yeast extract to 0.50 g/l and replace pyruvate with 0.50 g/l Na-acetate added from an anoxic sterile stock solution. Use 5% (v/v) as inoculum.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
FeCl ₂ x 4 H ₂ O	1.00	g
$MnCl_2 \times 4 H_2O$	0.10	g
CoCl ₂ x 6 H ₂ O	0.03	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnCl ₂	0.10	g
CuCl ₂	0.02	g
H ₃ BO ₃	0.01	g
$Na_2MoO_4 \times 2 H_2O$	0.03	g
$NiCl_2 \times 6 H_2O$	0.10	g
NaCl	1.00	g
$Na_2SeO_3 \times 5 H_2O$	0.03	g
$Na_2WO_4 \times 2 H_2O$	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 HCI	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