1494: . OMIZ-PAT Medium (modified)

This recipe contains strain-specific modifications for *Breznakiella homolactica* DSM 111054 *

Final pH: 6.9
Final volume: 200 ml

Distilled water	100.00	ml
Solution A	10.00	ml
Solution B	10.00	ml
Solution C	10.00	ml
Solution D	10.00	ml
Solution E	10.00	ml
Solution F	10.00	ml
Solution G	10.00	ml
Solution H	200.00	µl
Solution I	8.00	µl
Solution J	2.00	µl
Solution K	40.00	µl
Solution L	2.00	ml
Trace elements I (pre-dilute 1:1000; use)	2.00	ml
Trace elements II (pre-dilute 1:1000; use)	2.00	ml
Trace elements III (pre-dilute 1:1000; use)	200.00	µl
Distilled water (add up to 200 ml)	200.00	ml
Supplement 1	200.00	µl
Supplement 2	2.00	ml
Supplement 3	2.00	ml
Supplement 4	200.00	µl
Supplement 5	2.00	ml

1. Add 10 ml rabbit serum, flush the medium with N_2CO_2, adjust the pH to 6.9 and filter-sterilize the completed medium into tubes.

Rabbit serum	10.00	ml

2. This recipe describes the preparation of 200 ml medium, which can be used for about 4 weeks when kept at 4°C.

* plus 0.5 bar hydrogen

Solution A

L-Alanine	90.00	mg
L-Arginine	350.00	mg
L-Asparagine	300.00	mg
L-Aspartic acid	236.00	mg

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L-Cysteine HCl	700.00	mg
L-Glutamine	1360.00	mg
L-Glutamic acid	600.00	mg
Glycine	150.00	mg
L-Histidine	1240.00	mg
L-Isoleucine	262.00	mg
L-Leucine	262.00	mg
L-Lysine HCl	364.00	mg
L-Methionine	300.00	mg
L-Ornithine HCl	340.00	mg
L-Phenylalanine	330.00	mg
L-Proline	230.00	mg
L-Serine	1050.00	mg
L-Threonine	240.00	mg
L-Tryptophan	204.00	mg
L-Tyrosine	180.00	mg
L-Valine	234.00	mg
Distilled water	100.00	ml

Prepare Solutions A, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Solution B

KCl	1936.00	mg
$MgSO_4$ x 7 H_2O	500.00	mg
NaH_2PO_4 x H_2O	280.00	mg
NH_4Cl	3200.00	mg
Citric acid, trisodium salt	400.00	mg
Distilled water	100.00	ml

Prepare Solutions B, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Solution C

Calcium D-(+)-pantothenate	10.00	mg
Choline chloride	100.00	mg
Myo-inositol	100.00	mg
Thiamine HCl	10.00	mg
Thiamin pyrophosphate	10.00	mg
Coenzym A, sodium salt	2.00	mg
Flavin adenine dinucleotide (FAD)	2.00	mg
ß-nicotinamide adenine dinucleotide (NAD)	2.00	mg
2-Mercaptoethanesulfonic acid (MES), sodium salt	20.00	mg

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N-acetylmuramic acid	50.00	mg
N-Acetylglucosamine	400.00	mg
D-glucuronic acid, sodium salt	1600.00	mg
D-galacturonic acid	1600.00	mg
Pyruvic acid, sodium salt	1100.00	mg
Fumaric acid, disodium salt	1000.00	mg
Formic acid, sodium salt	600.00	mg
Distilled water	100.00	ml

Prepare Solutions C, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Solution D

Pyridoxal hydrochloride	10.00	mg
Pyridoxal phosphate	10.00	mg
D, L-lactic acid, sodium salt	1120.00	mg
Hypoxanthine	2.80	mg
Uracil	2.20	mg
Thymidine	4.80	mg
N-(2-acetamido)-2-aminoethanesulfonic acid	3.60	mg
DL-carnitine	400.00	mg
Putrescine 2 HCl	10.00	mg
Phenol red, sodium salt	20.00	mg
Distilled water	100.00	ml

Prepare Solutions D, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Solution E

D-Glucose	4.00	g
D-Fructose	1.60	g
D-Maltose	1.60	g
D-Mannitol	1.60	g
D-Mannose	4.00	g
D-Arabinose	4.00	g
L-Fucose	4.00	g
D-Trehalose	4.00	g
D-Sucrose	4.00	g
L-Rhamnose	4.00	g
Distilled water	100.00	ml

Prepare Solutions E, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

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Solution F

$CaCl_2$ x H_2O	300.00	mg
Distilled water	100.00	ml

Prepare Solution F, flush with nitrogen to make it anoxic, filter-sterilize and store at 4°C

Solution G

Asialofetuin	1.00	mg/ml

Prepare Solutions G, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Solution H

Vitamine B_{12}	5.00	mg
D-(+)-biotin	5.00	mg
Folic acid	5.00	mg
Folinic acid, calcium salt	10.00	mg
Nicotinamide	5.00	mg
Nicotinic acid	10.00	mg
Riboflavine	1.00	mg
Distilled water	100.00	ml

Prepare Solutions H, flush each with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Solution I

Isobutyric acid	10.00	µl
2-Methylbutyric acid	10.00	µl
Valeric acid	10.00	µl
Isovaleric acid	10.00	µl

Prepare Solution I and store at -20°C

Solution J

DL-alpha-lipoic acid	1.00	mg/ml
2-Mercaptoethanol	0.10	ml
Ethanol	0.90	ml

D,L-alpha-lipoic acid (1 mg/ml); in 2-mercaptoethanol-ethanol (1:10); store at -20°C

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Solution K

Hemin	0.20	mM
NaOH	10.00	mM

Hemin (0.2 mM) in NaOH (10 mM)

Solution L

Yeast extract (fresh)	42.00	g
Distilled water	140.00	ml

Homogenize 42 g fresh baker yeast in 140 ml distilled water, incubate for 24h at 56°C and centrifuge to harvest the supernatant. Adjust pH to 7 with NaOH, make anoxic, filter-sterilize and store at -80°C

Trace elements I

$CuSO_4$	7.89	mg
$MnSO_4$ x H_2O	169.00	mg
$ZnSO_4$ x 7 H_2O	2.87	g
HCl (10 mM)	100.00	ml

Prepare Trace elements I, flush with nitrogen to make them anoxic, filter-sterilize and store at 4°C

Trace elements II

Na_2SeO_3	173.00	mg
HCl (10mM)	100.00	ml

Prepare Trace elements II, flush with nitrogen to make them anoxic, filter-sterilize and store at 4°C

Trace elements III

$NiSO_4$ x 6 H_2O	13.10	mg
$SnCl_2$ x 2 H_2O	11.80	mg
$NaVO_3$	61.00	mg
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	1240.00	mg
HCl (10 mM)	100.00	ml

Prepare Trace elements III, flush with nitrogen to make them anoxic, filter-sterilize and store at 4°C

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Supplement 1

Fe SO_4 x 7 H_2O	10.00	mM
HCl	10.00	mM

1. $FeSO_4$ x 7H2O (10 mM) in HCl (10 mM)

2. Prepare Supplements 1, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Supplement 2

Ascorbic acid	10.00	%(w/v)
Distilled water	2.00	ml

1. Ascorbic acid (10%; w/v); in distilled water, neutralize with NaOH

2. Prepare Supplements 2, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Supplement 3

Glutathione	0.50	M
Distilled water	2.00	ml

1. Glutathion (0.5 M); in distilled water, neutralize with NaOH

2. Prepare Supplements 3, flush with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Supplement 4

Cholesterol	1.00	mg/ml
Ethanol (100 %)	1.00	ml

Cholesterol (1 mg/ml); in Ethanol (100%); store at -20°C

Supplement 5

$NaHCO_3$	10.00	%(w/v)
Distilled water	2.00	ml

$NaHCO_3$ (10%; w/v) in distilled water, store at 4°C

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