

1494 . OMIZ-PAT Medium (modified)

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

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

Solution A:

L-Alanine	90	mg
L-Arginine	350	mg
L-Asparagine	300	mg
L-Aspartic acid	236	mg
L-Cystein Hcl	700	mg
L-Glutamine	1360	mg
L-Glutamic acid	600	mg
Glycine	150	mg
L-Histidine	1240	mg
L-Isoleucine	262	mg
L-Leucine	262	mg
L-Lysine HCl	364	mg
L-Methionine	300	mg
L-Ornithine HCl	340	mg
L-Phenylalanine	330	mg
L-Proline	230	mg
L-Serine	1050	mg
L-Threonine	240	mg
L-Tryptophan	204	mg
L-Tyrosine	180	mg
L-Valine	234	mg
Distilled Water	100	ml

Solution B:

KCl	1936	mg
MgSO ₄ x 7H ₂ O	500	mg
NaH ₂ PO ₄ x H ₂ O	280	mg
NH ₄ Cl	3200	mg
Citric acid, trisodium salt	400	mg
Distilled water	100	ml

Solution C:

Calcium D-(+)-panthothenate	10	mg
Choline chloride	100	mg
Myo-Inositol	100	mg
Thiamine dichloride	10	mg
Thiamin pyrophosphate	10	mg
Coenzym A, sodium salt	2	mg
Flavin adenine dinucleotide (FAD)	2	mg
β -nicotinamide adenine dinucleotide (NAD)	2	mg
2-mercaptoethanesulfonic acid, sodium salt (MES)	20	mg
N-acetylmuramic acid	50	mg
N-acetylglucosamin	400	mg
D-glucuronic acid, sodium salt	1600	mg
D-galacturonic acid	1600	mg
Pyruvic acid, sodium salt	1100	mg
Fumaric acid, disodium salt	1000	mg
Formic acid, sodium salt	600	mg
Distilled water	100	ml

Solution D:

Pyridoxal - HCl	10	mg
Pyridoxal - Phosphat	10	mg
D,L-lactic acid, sodium salt	1120	mg
Hypoxanthine	2.8	mg
Uracil	2.2	mg
Thymidine	4.8	mg
N-(2-acetamido)-2-aminoethanesulfonic acid	3.6	mg
D,L-carnithine	400	mg
Putrescine 2 HCl	10	mg
Phenol red, sodium salt	20	mg
Distilled water	100	ml

Solution E:

D-Glucose	4	g
D-Fructose	1.6	g
D-Maltose	1.6	g
D-Mannitol	1.6	g
D-Mannose	4	g
D-Arabinose	4	g
L-Fucose	4	g
D-Trehalose	4	g

D-Sucrose	4	g
L-Rhamnose	4	g
Distilled water	100	ml

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

Solution F:

CaCl ₂ x H ₂ O	300	mg
Distilled water	100	ml

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

Solution G:

Asialofetuin (1 mg/ml) in distilled water

Solution H:

Vitamine B12	5	mg
D-(+)-biotin	5	mg
Folic acid	5	mg
Folinic acid, calcium salt	10	mg
Nicotinamide	5	mg
Nicotinic acid	10	mg
Riboflavine	1	mg
Distilled water	100	ml

Prepare **Solution I** and store at -20°C

Solution I:

Isobutyric acid	10	μl
2-methylbutyric acid	10	μl
Valeric acid	10	μl
Isovaleric acid	10	μl

Solution J:

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

Solution K:

Hemin (0.2 mM) in NaOH (10 mM)

Solution L:

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

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

Trace elements I

CuSO ₄	7.89	mg
MnSO ₄ x H ₂ O	169	mg
ZnSO ₄ x 7H ₂ O	2.87	g
10 mM HCl	100	ml

Trace elements II

Na ₂ SeO ₃	173	mg
10mM HCl	100	ml

Trace elements III

NiSO ₄ x 6H ₂ O	13.1	mg
SnCl ₂ x 2H ₂ O	11.8	mg
NaVO ₃	61	mg
(NH ₄) ₆ Mo ₇ O ₂₄ x H ₂ O	1240	mg
10 mM HCl	100	ml

Prepare **Supplements 1 - 3**, flush each with nitrogen to make them anoxic, filter-sterilize and store at -20°C

Supplement 1:

FeSO₄ x 7H₂O (10 mM) in HCl (10 mM)

Supplement 2:

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

Supplement 3:

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

Supplement 4:

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

Supplement 5:

NaHCO₃ (10%; w/v) in distilled water, store at 4°C

Preparation of medium

Distilled water		100	ml
Solution A-G		10	ml of each
Solution H		200	µl
Solution I		8	µl
Solution J		2	µl
Solution K		40	µl
Solution L		2	ml
Trace elements I and II	pre-dilute 1:1000; use	2	ml of each
Trace elements III	pre-dilute 1:1000; use	200	µl

Add distilled water to 200 ml

Supplements 2, 3, 5	2	ml of each
Supplements 1, 4	200	µl of each

Add 10 ml rabbit serum, flush the medium with N₂CO₂, adjust the pH to 6.9 and filter-sterilize the completed medium into tubes.