ATCC medium: 2131 OMIZ - P4

Basal Medium:
Add the ingredients below to 500.0 ml of distilled water in a 1.0 L volumetric flask:

Alanine.....45.0 mg
Arginine.....174.0 mg
Asparagine.....150.0 mg
Aspartic acid.....133.0 mg
L-Cysteine . HCl.....352.0 mg
Glutamic acid.....294.0 mg
Glutamine.....680.0 mg
Glycine.....75.0 mg
Histidine.....620.0 mg
Isoleucine.....131.0 mg
Leucine.....131.0 mg
Lysine HCl.....182.0 mg
Methionine.....149.0 mg
Ornithine HCl.....168.0 mg
D,L-Carnitine.....200.0 mg
Phenol red.....10.0 mg
2-Methylbutyric acid.....10.0 mcl
$CaCl_2$ Solution (see below).....1.0 ml
Ni-Sn-V-Mo Solution (see below)....1.0 ml
Phenylalanine.....165.0 mg
Proline.....115.0 mg
Serine.....525.0 mg
Threonine.....119.0 mg
Tryptophan.....102.0 mg
Tyrosine.....90.0 mg
Valine.....117.0 mg
KCl.....968.0 mg
$MgCl_2$. $6H_2O$ .....203.3 mg
NaH_2PO_4 . H_2O.....140.0 mg
NH_4Cl .....1.6 g
D-Galacturonic acid.....2.0 g
Hypoxanthine.....1.4 mg
Thymidine.....2.4 mg
Putrescine . 2HCl.....5.0 mg
Lactic acid (30%).....1.4 ml
Valeric acid.....10.0 mcl
Cu-Mn-Zn Solution (see below).....1.0 ml
Vitamin Solution (see below).....1.0 ml
D-Maltose.....2.0 g
D-Mannose.....2.0 g
D-Ribose.....2.0 g
D-Xylose.....2.0 g
L-Fucose.....2.0 g

Sodium citrate.....200.0 mg
N-Acetylglucosamine.....500.0 mg
Sodium pyruvate.....550.0 mg
Sodium fumarate.....500.0 mg
Sodium formate.....300.0 mg
Adenine.....1.4 mg
D-Arabinose.....2.0 g
D-Glucose.....2.0 g
Uracil.....1.1 mg
ACES.....1822.0 mg
Spermidine.....5.0 mg
Iso-butyric acid.....10.0 mcl
Iso-valeric acid.....10.0 mcl
Selenium Solution (see below).....1.0 ml
Hemin Solution (see below).....1.0 ml
Lipoic Acid Solution (see below)...1.0 ml

After all of the above ingredients have been added to 500.0 ml of distilled water, adjust pH to 6.0.

$CaCl_2$ Solution:
$CaCl_2 \cdot H_2O$ .....14.7 g
10mM HCl.....100.0 ml

Ni-Sn-V-Mo Solution:
$NiSO_4 \cdot 6H_2O$ .....0.131 mg
$SnCl_2 \cdot 2H_2O$ .....0.118 mg
$NaVO_3$.....0.61 mg
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$.....12.4 mg
10mM HCl.....1.0 L

Cu-Mn-Zn Solution:
$CuSO_4$.....0.798 mg
$MnSO_4 \cdot H_2O$ .....0.169 mg
$ZnSO_4 \cdot 7H_2O$ .....0.287 g
10mM HCl.....1.0 L

Vitamin Solution:
Calcium D-(+)-pantothenate.....500.0 mg
Thiamine-2HCl.....500.0 mg
Pyridoxal phosphate.....500.0 mg
Folinic acid, Calcium salt.....100.0 mg
Riboflavin.....1.0 mg
Coenzyme A, sodium.....100.0 mg
Choline chloride.....5.0 g
Thiamine pyrophosphate.....2.5 g
D(+)-Biotin.....5.0 mg
Nicotinamide.....50.0 mg
Vitamin B12.....5.0 mg

FAD.....100.0 mg
myo-Inositol.....5.0 g
Pyridoxal-HCl.....500.0 mg
Folic acid.....5.0 mg
Nicotinic acid.....100.0 mg
beta-NAD.....100.0 mg
2-Mercaptoethanesulfonic acid.....1.0 g
Distilled water.....100.0 ml

Lipoic Acid Solution:
DL-alpha-Lipoic acid.....10.0 mg
2-Mercaptoethanol.....1.0 ml
Ethanol.....9.0 ml

Filter-sterilize.

Selenium Solution:
$NaSeO_3$.....17.3 mg
10mM HCl.....1.0 L

Hemin Solution:
Hemin.....12.5 mg
10mM NaOH.....100.0 ml

Prior to use, add the following solutions, then bring the volume to 1.0 L. Adjust pH to 6.9. Filter-sterilize.

Sodium Bicarbonate Solution (see below).....10.0 ml
DHNA Solution (see below).....1.0 ml
Heat-inactivated human serum (Sigma H-1388)...10.0 ml
Vitamin C Solution (see below).....10.0 ml
Cholesterol Solution (see below).....1.0 ml
Glutathione Solution (see below).....10.0 ml
Yeast Extract Solution (Gibco 18180-059).....20.0 ml
Ferrous Sulfate Solution (see below).....1.0 ml
Neopeptone Solution (see below).....10.0 ml

Sodium Bicarbonate Solution:
$NaHCO_3$.....10.0 g
Distilled water.....100.0 ml

Filter-sterilize.

DHNA Solution:
1,4-Dihydroxy-2-naphthoic acid....40.8 mg
Ethanol.....100.0 ml

Filter-sterilize.

Vitamin C Solution:
Vitamin C.....10.0 g
Distilled water.....70.0 ml

Neutralize with NaOH.
Bring volume to 100.0 ml
Filter-sterilize.

Cholesterol Solution:
Cholesterol.....20.0 mg
Ethanol.....20.0 ml

Filter-sterilize.

Glutathione Solution:
Glutathione, reduced.....30.73 g
Distilled water.....150.0 ml

Neutralize with NaOH.
Bring volume to 200.0 ml
Filter-sterilize.

Ferrous Sulfate Solution:
$FeSO_4 \cdot 7H_2O$ .....278.0 mg
10mM HCl.....100.0 ml

Filter-sterilize.

Neopeptone Solution:
Neopeptone, DIFCO (BD 211681).....10.0 g
Distilled water.....100.0 ml

Filter-sterilize.

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