Media preparation

Tissue culture medium consists of many items that are classified into micro- and macroelements, carbon source, vitamins, amino acids, growth regulators and undefined supplements. Many formulations are now available for minerals and vitamins of which Murashige and Skoog (1962) medium (MS medium) is the most commonly used medium in tissue culture labs. To avoid weighing and dissolving each of these items whenever we are going to prepare tissue culture medium, most of these items are prepared as separate concentrated stock solutions that are mixed and diluted with water before use.

How to prepare and use stock solution

To Prepare A ml stock soln (B X)

Dissolve (Concn in medium (mg/ml) X A X B) mg In total volume A ml.

Ex1: 50 ml Magnesium Sulfate Stock Solution (100X)

Each liter of MS medium contains 370 mg $MgSO_4$ .7 H_2O

i.e. each ml medium contains 370 / 1000 = 0.37 mg

Dissolve (0.37 X 50 X 100) = 1850 mg in total volume 50 ml.

The volume (V ml) required from this stock to prepare V' ml Ms medium is calculated according to the following equation:

$$NV=N'V'$$

N is the concentration (expressed in any unit i.e X, mg/L or mg/ml) of $MgSO_4$.7 H_2O in stock.

N' is the required concentration (expressed in the same unit used with N) of $MgSO_4$.7 H_2O in medium.

V is the volume (expressed in L or ml) of $MgSO_4 .7 H_2O$ stock required.

V' is the volume (expressed in the same unit used with V) of medium to be prepared.

If we want to prepare 1000 ml medium we need

V ml of stock = (N'V')/N = (0.37 X 1000) / 37 = 10.0 ml

Ex2: 100 ml Vitamins (Thiamine, Pyridoxine and Nicotinic Acid) Stock Solution (1000X)

Thiamine

Each liter MS medium contains 0.1 mg

i.e. each ml medium contains 0.0001 mg.

Wt required for stock = 0.0001 X 100 X 1000 = 10 mg

Pyridoxine

Each liter MS medium contains 0.5 mg

i.e. each ml medium contains 0.0005 mg.

Wt required for stock = 0.0005 X 100 X 1000 = 50 mg

Nicotinic Acid

Each liter MS medium contains 0.5 mg

i.e. each ml medium contains 0.0005 mg.

Wt required for stock = 0.0005 X 100 X 1000 = 50 mg

Dissolve 10 mg Thiamine, 50 mg Pyridoxine and 50 mg Nicotinic Acid in 100 ml total volume.

If we want to prepare 1000 ml medium we need

Based on thiamine

V ml of stock = (N'V')/N = (0.0001 X 1000)/0.1 = 1.0 ml

Minerals, either alone or along with some vitamins, are available as powder or liquid from which certain amount are dissolved or diluted with water to prepare any amount of medium.

Stock Solutions for Growth Regulators

1 mg/ml = 1000 mg/L

X mg in **X** ml total solution

1 µM/ml (1µM = Mol Wt / 1 000 000)

X µM in **X** ml total solution

How to Use Stock Solutions for Growth Regulators

N V = N' V'

Ex: Preparation of 1 liter medium containing 4.5 mg/L BA. Using stock solution (1 mg/ml):

Each liter of medium contains 4.5 mg

i.e. each ml medium contains 4.5 / 1000 = 0.0045 mg

Concn. of medium = 4.5 mg/L OR 0.0045 mg/ml

Each liter stock contains 1000 mg

i.e. each ml stock contains 1000 / 1000 = 1 mg

Concn. of stock = 1000 mg/L OR 1 mg/ml

V ml of stock = (N'V')/N = (0.0045 X 1000)/1 = 4.5 ml

Compounds	Murashige and Skoog	Gamborg B-5	WPM	Nitsch and Nitsch	Schenk and Hildebrandt	White
Macronutrients in mg/L (mM)						
NH_4NO_3	1650 (20.6)	—	400 (5.0)	—	—	—
$NH_4H_2PO_4$	—	—	—	—	300 (2.6)	—
NH_4SO_4	—	134 (1.0)	—	—	—	—
$CaCl_2 \cdot 2H_2O$	332.2 (2.3)	150 (1.0)	96 (0.7)	166 (1.1)	151 (1.0)	—
$Ca(NO_3)_2 \cdot 4H_2O$	—	—	556 (2.4)	—	—	288 (1.2)
$MgSO_4 \cdot 7H_2O$	370 (1.5)	250 (1.0)	370 (1.5)	185 (0.75)	400 (1.6)	737 (3.0)
KCl	—	—	—	—	—	65 (0.9)
KNO_3	1900 (18.8)	2500 (24.8)	—	950 (9.4)	2500 (24.8)	80 (0.8)
K_2SO_4	—	—	990	—	—	—
KH_2PO_4	170 (1.3)	—	170 (1.3)	68 (0.5)	—	—
NaH_2PO_4	—	130.5 (0.9)	—	—	—	16.5 (0.12)
Na_2SO_4	—	—	—	—	—	200 (1.4)
Micronutrients in mg/L (mM)						
H_3BO_3	6.2 (100)	3.0 (49)	6.2 (100)	10 (162)	5 (80)	1.5 (25)
$CoCl_2 \cdot 6H_2O$	0.025 (0.1)	0.025 (0.1)	—	—	0.1 (0.4)	—
$CuSO_4 \cdot 5H_2O$	0.025 (0.1)	0.025 (0.1)	0.25 (1)	0.025 (0.1)	0.2 (0.08)	0.01 (0.04)
Na_2EDTA	37.3 (100)	37.3 (100)	37.3 (100)	37.3 (100)	20.1 (54)	—
$Fe_2(SO_4)_3$	—	—	—	—	—	2.5 (6.2)
$FeSO_4 \cdot 7H_2O$	27.8 (100)	27.8 (100)	27.8 (100)	27.8 (100)	15 (54)	—
$MnSO_4 \cdot H_2O$	16.9 (100)	10.0 (59)	22.3 (132)	18.9 (112)	10.0 (59)	5.04 (30)
KI	0.83 (5)	0.75 (5)	—	—	0.1 (0.6)	0.75 (5)
$NaMoO_3$	—	—	—	—	—	0.001 (0.001)
$Na_2MoO_4 \cdot 2H_2O$	0.25 (1)	0.25 (1)	0.25 (1)	0.25 (1)	0.1 (0.4)	—
$ZnSO_4 \cdot 7H_2O$	8.6 (30)	2.0 (7.0)	8.6 (30)	10 (35)	1 (3)	2.67 (9)
Organics in mg/L (mM)						
Myo-inositol	100 (550)	100 (550)	100 (550)	100 (550)	1000 (5500)	—
Glycine	2.0 (26.6)	—	2.0 (26.6)	2.0 (26.6)	—	3.0 (40)
Nicotinic acid	0.5 (4.1)	1.0 (8.2)	0.5 (4.1)	5 (40.6)	5.0 (41)	0.5 (4.1)
Pyridoxine HCl	0.5 (2.4)	0.1 (0.45)	0.5 (2.4)	0.5 (2.4)	0.5 (2.4)	0.1 (0.45)
Thiamin HCl	0.1 (0.3)	10.0 (30)	1.0 (3.0)	0.5 (1.5)	5.0 (14.8)	0.1 (0.3)
Biotin	—	—	—	0.2 (0.05)	—	—

Q1: MS medium contains 332.2 mg/L $CaCl_2 . 2H_2O$ and 1900 mg/L KNO_3

Describe how to make 250 ml stock solution (50 X) containing both substances.

Calculate the volume required from this stock to prepare 750 ml **MS** medium.

Calculate the volume required from this stock to prepare 750 ml **B_5** medium.

Q2: You are provided with:

1. **powdered MS medium (salts and vitamins)**
2. **Sucrose**
3. **myo-inositol stock solution (10 mg/ml).**
4. **BA (1 mg/ml)**
5. **IAA (1 mg/ml)**
6. **Agar**

Describe preparation of 250 ml MS medium *(salts and vitamins)* supplemented 30 g/L sucrose, 100 mg/L myo-inositol, BA (2, 4 or 6 mg/L either alone or in combination with 0.1 mg/L IAA) and 8 g/L agar. The pH is 5.8.