

Appendix 1. Media and solution composition

MS medium (Murashige and Skoog 1962)

MS components	Concentration (mg/L)
Inorganic salts	
Calcium Chloride	332.02
Ammonium Nitrate (NH_4NO_3)	1650
Magnesium Sulfate	80.70
Boric Acid (H_3BO_3)	6.2
Cobalt Chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$)	0.025
Cupric Sulfate ($\text{CuSO}_4 \cdot 6\text{H}_2\text{O}$)	0.025
Manganese Sulfate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$)	16.90
Potassium Iodide (KI)	0.83
Potassium Nitrate (KNO_3)	1900
Potassium Phosphate (KH_2PO_4)	170
Sodium Molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)	0.25
Zinc Sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	8.60

Iron source

Sodium EDTA ($\text{Na}_2 \cdot \text{EDTA}$)	37.26
Ferric Sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	27.80

Vitamins

Myo-inositol	100
Nicotinic Acid	0.5
Pyridoxine HCL	0.5
Thiamine HCl	0.5
Glycine (free base)	2.00

Vitamins of Morel (Morel 1950)

Calcium panthotenate	1
Myo-inositol	100
Nicotinic Acid	1
Pyridoxine HCL	1
Thiamine HCl	1
Biotine	0.01

P5 medium

MS medium supplemented with sucrose 30 g/L, 10 μ M BA, 1 μ M IAA, 2 g/L gelrite or 5 g/L agar (Banerjee and De Langhe 1985). (pH 5.8).

P4 medium

P5 medium with a 10-fold higher (100 μ M) BAP concentration.

Preculture medium (PCM)

This medium contains all P5 elements but the sucrose level is increased to a final concentration of 0.4 M (=136.8 g/L).

P6 Regeneration medium

P5 medium with a 10-fold-lower (1 μ M) BAP concentration.

Loading solution

MS medium components diluted in water supplemented with 2 M glycerol and 0.4 M (=136.8 g/L) sucrose, pH adjusted to 5.8. The solution is sterilized through a filter (0.22 μ m).

PVS2 solution

Consists of 30% (w/v) (3.26 M) glycerol, 15% (w/v) (2.42 M) ethylene glycol (w/v) (EG), 15% (w/v) (1.9 M) DMSO and 0.4 M (= 136.8 g/L) sucrose (Sakai et al. 1990). All these compounds are dissolved in MS medium, pH adjusted to 5.8 followed by filter sterilization (0.22 μ m).

Unloading solution

The filter sterilized (0.22 μ m) unloading solution consists of 1.2 M (410.4 g/L) sucrose dissolved in MS medium. (pH 5.8).

ZZ medium

Half strength MS macroelements and iron, MS microelements, 5 μ M 2,4-D, 1 μ M zeatine, standard MS vitamins, 10 mg/L ascorbic acid, and 30 g/L sucrose (pH 5.8).

RD1 medium

MS macroelements and iron, MS microelements, 1 μ m BA, standard MS vitamins, 100 mg/L myo-inositol, 10 mg/L ascorbic acid, 30 g/L sucrose and 2 g/L gelrite. (pH 5.8).

MA2 medium

MS macro- and micro-elements, biotine 1 mg/L, glutamine 100 mg/L, malt extract 100 mg/L, 2,4-D 1 mg/L and sucrose 45 g/L. (pH 5.3).

MA3 medium

Inorganic salts	Concentration (mg/L)
KNO ₃	2500
CaCl ₂ · 2H ₂ O	200
MgSO ₄ · 7 H ₂ O	400
NH ₄ H ₂ PO ₄	300
MnSO ₄ · H ₂ O	10
H ₃ BO ₃	5
ZnSO ₄ · 7H ₂ O	1
KI	1
CuSO ₄ · 5H ₂ O	0.2
NaMoO ₄ · 2H ₂ O	0.1
CoCl ₂	0.1

Iron source

FeSO ₄ · 7H ₂ O	15
Na ₂ DTA	20

MS vitamins**Other components**

ANA	0.2
Zeatine	0.05
2iP	0.2
Kinetine	0.1
Lactose	10 g/L
Sucrose	45 g/L
Agarose	7 g/L

pH 5.3