

Yam Embryo Germination Media (YEGM)

	[final]	[stock]	prep / L	/ 250 mL
Sucrose	30 g/L	--	30 g	7.5 g
MS Macronutrients (10x)			100 mL	25 mL
NH ₄ NO ₃ (ammonium nitrate)	1.65 g/L	16.5 g/L		
KNO ₃ (potassium nitrate)	1.9 g/L	19 g/L		
CaCl ₂ ·2H ₂ O ^(A)	0.44 g/L	4.4 g/L ^(A)		
MgSO ₄ ·7H ₂ O ^(B)	0.37 g/L	3.7 g/L ^(B)		
KH ₂ PO ₄ (potassium phosphate monobasic)	0.17 g/L	1.7 g/L		
MS Micronutrients (1000x)			1 mL	250 µL
MnSO ₄ ·H ₂ O ^(C) (manganous sulfate)		16.9 g/L ^(C)		
ZnSO ₄ ·7H ₂ O		8.6 g/L		
H ₃ BO ₃ (boric acid)		6.2 g/L		
KI (potassium iodide)		0.83 g/L		
Na ₂ MoO ₄ ·2H ₂ O (sodium molybdate)		0.25 g/L		
CuSO ₄ ·5H ₂ O ^(D)		0.025 g/L ^(D)		
CoCl ₂ ·6H ₂ O		0.025 g/L		
Fe-EDTA ·[2H ₂ O] ^(E) (iron-EDTA)	40.3 mg/L	4.0 g/L (4.0 mg/mL)	10 mL	2.5 mL
MS vitamins Thiamine-HCl	0.1 mg/L	0.1 g/L (0.1 mg/mL)	1 mL	250 µL
Nicotinic acid	0.5 mg/L	0.5 g/L (0.5 mg/mL)		
Pyridoxine	0.5 mg/L	0.5 g/L (0.5 mg/mL)		
Inositol	100 mg/L	10 g/L (10 mg/mL)	10 mL	2.5 mL
Glycine	2 mg/L	0.2 g/L (0.2 mg/mL)	10 mL	2.5 mL
pH = 5.7 (record initial pH)				
Adams Scientific Gelrite for Plant TC	6.5 g/L	--	6.5 g	1.63 g
Autoclave				
6-Benzylaminopurine (BAP)	0.4mg/L	10mg/mL	40 uL	10 uL

- (A) (dihydrate MW=147) If use anhydrous CaCl₂ (MW=111), stock contains 3.32 g/L
- (B) (heptahydrate MW=246.5) If use anhydrous MgSO₄ (MW=120), stock contains 1.81 g/L
- (C) (manganese II sulfate, monohydrate MW=169.0) If use anhydrous MnSO₄ (MW=151), stock contains 15.1 g/L. Many people polder formulations specify MnSO₄·4H₂O (could not find in catalogs), stock 22.3 g/L.
- (D) (pentahydrate MW=249.7) If use anhydrous CuSO₄ (MW=159.6), stock contains 0.016 g/L.
- (E) (Ferrous EDTA: FeNaC₁₀H₁₂N₂O₈; anhydrous MW=367.1) The original Iron-chelator solution was created by adding 27.3 mg/L FeSO₄·7H₂O (FW=278) plus 37.3 mg/L Na₂-EDTA [although the degree of hydration of di-sodium EDTA is not specified on several venders (Sigma, Gibco) the dihydrate (FW=372.2) is the only available form, and this would give the same molarity (0.100 mM) for both Fe and EDTA. The iron-sodium-EDTA is now available, but apparently it has a variable degree of hydration (3 batches - all were different). Equivalent Fe and EDTA molarity can be calculated: Fe-EDTA·[2H₂O] (FW=403.1), 4.03 g/L; [·2.5H₂O] (FW=412.1), 4.12 g/L; [·3H₂O] (FW=421.1), 4.21 g/L ...

Application:

1) General salts formulation (with both ammonia and nitrate nitrogen source) that is the basic stock (and often default) for many specialized media (regeneration, transformation, micropropagation ...).

Procedure notes:

MS salts from basic solubility groups was the method used for Ph.D. This approach was abandoned '95-'01 since very little media was MS-based. With the increased use of tobacco, maintenance of shoot cultures, transgenic plants and media using MS, we went back to solution-based media prep using stock solutions 8/8/2001. Although I did not have the original stock solution protocols handy from Ph.D., the media stocks were based on examining numerous protocols for media.

See notes for B5 media for more details on Fe-EDTA stock solution.

Media Reference:

Murashige, Toshio//Skoog, Folke

A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497, 1962.