

Waris-H + 3V

Components and Final Concentration in Culture Medium	Stock Solution	Addition per Litre of Culture Medium
1. HEPES (1.00 mM)	238.10 g / l dH ₂ O	1 ml
2. KNO ₃ (1.00 mM)	100.00 g / l dH ₂ O	1 ml
3. MgSO ₄ x 7 H ₂ O (81.1 μM)	20.00 g / l dH ₂ O	1 ml
4. (NH ₄) ₂ HPO ₄ (0.15 mM)	20.00 g / l dH ₂ O	1 ml
5. Ca(NO ₃) ₂ x 4 H ₂ O (0.42 mM)	100.00 g / l dH ₂ O	1 ml
6. Vitamin Solution		3 ml
Vitamin B12 (0.15 nM)	0.20 mg / l dH ₂ O	
Biotin (4.10 nM)	1.00 mg / l dH ₂ O	
Thiamine-HCl (0.30 μM)	100.00 mg / l dH ₂ O	
Niacinamide (0.80 nM)	0.10 mg / l dH ₂ O	
pH of the Vitamin Solution should be around pH 7		
7. P-II Metals		1 ml
EDTA (Titriplex III) (8.06 μM)	3.00 g / l dH ₂ O	
H ₃ BO ₃ (18.43 μM)	1.14 g / l dH ₂ O	
MnCl ₂ x 4 H ₂ O (0.73 μM)	144.00 mg / l dH ₂ O	
ZnSO ₄ x 7 H ₂ O (73.00 nM)	21.00 mg / l dH ₂ O	
CoCl ₂ x 6 H ₂ O (16.80 nM)	4.00 mg / l dH ₂ O	
Dissolve EDTA and boric acid in dH ₂ O, then add metals one after the other.		
8. Fe-EDTA		1 ml
EDTA (Titriplex II) (17.86 μM)	5.22 g / l dH ₂ O	
FeSO ₄ x 7 H ₂ O (17.90 μM)	4.98 g / l dH ₂ O	
1 N KOH	54.00 ml / l dH ₂ O	
EDTA (Titriplex II) and FeSO ₄ x 7 H ₂ O is heated for 30 min (100°C); KOH is added to the cooled mixture.		
9. Soil extract		10 ml
adjust the pH to 7.0 and autoclave		

Preparation of Soil Extract

10 g of garden-soil is mixed with 120 ml dH₂O and boiled for 10 minutes. Afterwards it is centrifuged for 10 minutes (low speed), and the supernatant is filtered through a series of membrane filters from 1.2 μm – 0.1 μm pore size. The remaining filtrate is adjusted to 100 ml with dH₂O. Aliquots of 10 ml are stored frozen.

The soil should not be recently fertilized and should not contain too much humus.