Waris-H

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_2O	1 ml
2. **KNO_3** (1.00 mM)	100.00 g / l dH_2O	1 ml
3. **$MgSO_4$ x 7 H_2O** (81.1 µM)	20.00 g / l dH_2O	1 ml
4. **$(NH_4)_2HPO_4$** (0.15 mM)	20.00 g / l dH_2O	1 ml
5. **$Ca(NO_3)_2$ x 4 H_2O** (0.42 mM)	100.00 g / l dH_2O	1 ml
6. **Vitamin Solution**		1 ml
Vitamin B12 (0.15 nM)	0.20 mg / l dH_2O	
Biotin (4.10 nM)	1.00 mg / l dH_2O	
Thiamine-HCl (0.30 µM)	100.00 mg / l dH_2O	
Niacinamide (0.80 nM)	0.10 mg / l dH_2O	
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_2O	
H_3BO_3 (18.43 µM)	1.14 g / l dH_2O	
$MnCl_2$ x 4 H_2O (0.73 µM)	144.00 mg / l dH_2O	
$ZnSO_4$ x 7 H_2O (73.00 nM)	21.00 mg / l dH_2O	
$CoCl_2$ x 6 H_2O (16.80 nM)	4.00 mg / l dH_2O	
Dissolve EDTA and boric acid in dH_2O, then add metals one after the other.		
8. **Fe-EDTA**		1 ml
EDTA (Titriplex II) (17.86 µM)	5.22 g / l dH_2O	
$FeSO_4$ x 7 H_2O (17.90 µM)	4.98 g / l dH_2O	
1 N KOH	54.00 ml / l dH_2O	
EDTA (Titriplex II) and $FeSO_4$ x 7 H_2O 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.