ASP-12 (modified)

Components and Final Concentration in Culture Medium	Stock Solution	Addition per Litre of Culture Medium
1. **HEPES** (3.00 mM)	238.10 g / l dH_2O	3 ml
2. **NaCl** (479.00 mM)		weigh and add 28 g
3. **KCl** (9.40 mM)	60.00 g / l dH_2O	11.6 ml
4. **$MgSO_4$ x 7 H_2O** (28.40 mM)		weigh and add 7 g
5. **$MgCl_2$ x 6 H_2O** (19.70 mM)		weigh and add 4 g
6. **$CaCl_2$ x 2 H_2O** (10.00 mM)	370.00 g / l dH_2O	4 ml
7. **$NaNO_3$** (1.18 mM)	100.30 g / l dH_2O	1 ml
8. **K_3PO_4 x 3 H_2O** (47.00 µM)	12.50 g / l dH_2O	1 ml
9. **Na_2-Glycerophosphate** (31.70 µM)	6.85 g / l dH_2O	1 ml
10. **Na_2SiO_3 x 9 H_2O** (528.00 µM)	28.42 g / l dH_2O	5.3 ml
11. **NTA** (Titriplex I) (523.00 µM)	10.00 g / l dH_2O	10 ml
12. **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 7.0

13. **Trace Metals**

Na_2EDTA x 2 H_2O: 4.36 g

$FeCl_3$ x 6 H_2O: 3.15 g

Dissolve in 1000 ml dH_2O, then add 1 ml of Primary Trace Metals each (see below).

Primary Trace Metals are stored frozen as 1 ml aliquots.

13.2. **Primary Trace Metals**

13.2.1. K_2CrO_4	0.194 g / 100 ml dH_2O
13.2.2. $CoCl_2$ x 6 H_2O	1.00 g / 100 ml dH_2O
13.2.3. $CuSO_4$ x 5 H_2O	0.25 g / 100 ml dH_2O
13.2.4. $MnCl_2$ x 4 H_2O5	18.00 g / 100 ml dH_2O
13.2.5. Na_2MoO_4 x 2 H_2O	1.89 g / 100 ml dH_2O
13.2.6. $NiSO_4$ x 6 H_2O	0.27 g / 100 ml dH_2O
13.2.7. H_2SeO_3	0.13 g /100 ml dH_2O
13.2.8. Na_3VO_4	0.184 g /100 ml dH_2O
13.2.9. $ZnSO_4$ x 7 H_2O	2.20 g / 100 ml dH_2O

14. **Soil Extract** (optional)	See below	10 ml

adjust the pH to 7,9 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.