

## ASP-H (modified)

Components and Final Concentration in Culture Medium	Stock Solution	Addition per 1 Litre Culture Medium
1. HEPES (3.00 mM)	238.10 g / l dH <sub>2</sub> O	3 ml
2. NaCl (308.00 mM)		18 g
3. MgSO <sub>4</sub> x 7 H <sub>2</sub> O (20.3 mM)		5 g
4. KCl (8.10 mM)	60.00 g / l dH <sub>2</sub> O	10 ml
5. CaCl <sub>2</sub> x 2 H <sub>2</sub> O (2.50 mM)	370.00 g / l dH <sub>2</sub> O	1 ml
6. NaNO <sub>3</sub> (0.59 mM)	50.00 g / l dH <sub>2</sub> O	
K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O (22.00 μM)	5.00 g / l dH <sub>2</sub> O	
Na <sub>2</sub> CO <sub>3</sub> (0.30 mM)	30.00 g / l dH <sub>2</sub> O	1 ml
7. NTA (Titriplex I) (52.00 μM)	10.00 g / l dH <sub>2</sub> O	1 ml
NTA dissolves after addition of NaOH pellets and heating.		
8. Fe-EDTA		1 ml
EDTA (Titriplex II) (17.86 μM)	5.22 g	
FeSO <sub>4</sub> x 7 H <sub>2</sub> O (17.90 μM)	4.98 g	
1 N KOH	54.00 ml	
EDTA (Titriplex II) and FeSO <sub>4</sub> x 7 H <sub>2</sub> O is heated for 30 min (100°C); KOH is added to the cooled mixture.		
9. Trace Metals		1 ml
ZnCl <sub>2</sub> (2.40 μM)	0.33 g	
MnCl <sub>2</sub> x 4 H <sub>2</sub> O (21.90 μM)	4.30 g	
CoCl <sub>2</sub> x 6 H <sub>2</sub> O (0.51 μM)	0.12 g	
Na/EDTA (Titriplex III) (69.30 μM)	25.80g	
pH of Trace Metal Solution is adjusted to 7.5 with 10 N NaOH.		
10. H <sub>3</sub> BO <sub>3</sub> (0.48 mM)	2.97 g / l dH <sub>2</sub> O	10 ml
11. Vitamin Solution		1 ml
Vitamin B <sub>12</sub> (0.15 nM)	0.20 mg / l dH <sub>2</sub> O	
Biotin (4.10 nM)	1.00 mg / l dH <sub>2</sub> O	
Thiamine-HCl (0.30 μM)	100.00 mg / l dH <sub>2</sub> O	
Niacinamide (0.80 nM)	0.10 mg / l dH <sub>2</sub> O	

pH of this solution should be around pH 7.0

adjust the pH to 7,7 and autoclave