

1420. HALF STRENGTHS HYPHOMICROBIUM MEDIUM**Mineral Salts Solution A:**

KH_2PO_4	0.65	g
$\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$	1.34	g
Methylamine-HCl	1.69	g
Distilled Water	50.00	ml

Adjust pH to 7.5

Mineral Salts Solution B:

$(\text{NH}_4)_2\text{SO}_4$	0.250	g
$\text{MgSO}_4 \times 7\text{H}_2\text{O}$	0.100	g
Bacto Peptone	0.125	g
Trace Element Solution (see below)	1.000	ml
Hutner`s Basal Salts Solution (see below)	10.000	ml
Vitamine Solution (add after autoclaving, see below)	5.000	ml
Vitamin B ₁₂ (0.1%)(add after autoclaving)	1.250	µl
Distilled Water	1000.00	ml

Adjust pH to 7.5

For solid media add 15.0 g agar to solution B

Autoclave solution A and B separately and combine after cooling.

Trace Element Solution:

$\text{CaCl}_2 \times 2\text{H}_2\text{O}$	0.199	g
$\text{FeSO}_4 \times 7\text{H}_2\text{O}$	0.100	g
$\text{MnSO}_4 \times \text{H}_2\text{O}$	0.035	g
$\text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$	0.050	g
Distilled Water	100.00	ml

Hutner's salts:

Nitritotriacetic acid (NTA)	10.000	g
$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$	29.700	g
$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$	3.335	g
$(\text{NH}_4)_6\text{MoO}_7\text{O}_{24} \times 4 \text{H}_2\text{O}$	9.250	mg
$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$	99.000	mg
"Metals 44"	50.000	ml
Distilled water	950.000	ml

Dissolve the nitritotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H_2SO_4 .

"Metals 44":

Na-EDTA	250.000	mg
ZnSO ₄ x 7 H ₂ O	1095.000	mg
FeSO ₄ x 7 H ₂ O	500.000	mg
MnSO ₄ x H ₂ O	154.000	mg
CuSO ₄ x 5 H ₂ O	39.200	mg
Co(NO ₃) ₂ x 6 H ₂ O	24.800	mg
Na ₂ B ₄ O ₇ x 10 H ₂ O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of the heavy metal ions.

Vitamin solution:

Biotin	2.000	mg
Folic acid	2.000	mg
Pyridoxine-HCl	10.000	mg
Thiamine-HCl x 2 H ₂ O	5.000	mg
Riboflavin	5.000	mg
Nicotinic acid	5.000	mg
D-Ca-pantothenate	5.000	mg
Vitamin B ₁₂	0.100	mg
p-Aminobenzoic acid	5.000	mg
Distilled water	1000.000	ml

Prepare the liquid medium. Before inoculation add from sterile stock solutions:

Vitamin Solution	5.00	ml
Vitamin B ₁₂ (0.1%)	1.25	µl