

1229. THIOHALOSPIRA ALKALIPHILA MEDIUM

Mineral base:

NaCl	120.0	g
K ₂ HPO ₄	2.0	g
(NH ₄) ₂ SO ₄	0.5	g
Distilled water	1000.0	ml

Flush the gas phase with nitrogen and add 5% oxygen. Sterilize at 120°C 20 min in a closed vessel (i.e. a serum tube or bottle) with a liquid to air ratio of about 1:10.

Trace element solution:

EDTA	5.0	mg
FeSO ₄ x 7 H ₂ O	2.0	mg
ZnSO ₄ x 7 H ₂ O	100.0	mg
MnCl ₂ x 4 H ₂ O	30.0	mg
CoCl ₂ x 6 H ₂ O	200.0	mg
NiCl ₂ x 6 H ₂ O	20.0	mg
Na ₂ MoO ₄ x 2 H ₂ O	30.0	mg
CuCl ₂ x 2 H ₂ O	10.0	mg
H ₃ BO ₃	300.0	mg
Distilled water	1000.0	ml

Final pH should be 3, add HCl if needed. Sterilization - 120°C 20 min.

After sterilization add from sterile stock solutions:

Trace elements solution	1	ml
MgCl ₂ x 6 H ₂ O (200.0 g/l)	2	ml
<i>(a white colloid will form which will rapidly dissolve after mixing)</i>		
Sodium thiosulfate pentahydrate (4 M - 992 g/l) -	5	ml
Vitamin B12 (filter sterilized 100mg/l)	1	ml
NaHCO ₃ (84.0 g/l)	100	ml

Final pH is 8.0 – 8.5.

Incubate statically until growth occurs, then shake at 100 rpm.