

ATCC Medium 2768: Modified PYNFH in Seawater

Mix ATCC medium 1525 and ATCC medium 1405 at a 1:4 ratio
Supplement the above mixture with 5% complete ATCC medium 1034

Seawater Sonneborn's Paramecium Medium 1525.....	200ml
HESNW Medium 1405.....	800ml
PYNFH Medium 1034	50ml

1525

Seawater Sonneborn's Paramecium Medium

2X Seawater (Seven Seas)	500.0	ml
*2X Modified ATCC Medium 802	500.0	ml

*Filter-sterilize seawater.
Add seawater to cooled autoclaved 2X Modified ATCC Medium 802.*

*2X Modified ATCC Medium 802

Rye Cerophyl	5.0	g
Distilled water	1000.0	ml

*Add rye cerophyl to distilled water and boil for 5 minutes.
Add distilled water to compensate for evaporation.
Filter through Whatman #1 filter paper.
Autoclave at 121 C for 15 minutes.*

1405 HESNW Medium

Enrichment Solution	10.0	ml
Vitamin Solution	1.0	ml
Seawater	1000	ml

Combine ingredients. Filter sterilize.

Seawater

Sea Salts (Instant Ocean)	40.0	g
DI Water	1000	ml

Enrichment Solution

Na-EDTA	0.55	g
NaNO3	4.67	g
Na2SiO3 x 9 H2O	3.0	g
Na-Glycerophosphate	0.67	g
H3BO3	0.38	g
Fe(NH4)2(SO4)2 x 6H2O	0.23	g
FeCl3 x 6H2O	0.016	g
MnSO4 x 4H2O	0.054	g

ZnSO ₄ x 7 H ₂ O	7.3	mg
CoSO ₄ x 7H ₂ O	1.6	mg
DI Water	1000	ml

Vitamin Solution

Thiamine	0.1	g
Vitamin B12	2.0	mg
Biotin	1.0	mg
DI Water	1000	ml

ATCC Medium: 1034 PYNFH Medium

Peptone.....	10.0	g
Yeast Extract.....	10.0	g
Ribonucleic Acid (from Torula yeast).....	1.0	g
Folic Acid.....	15.0	mg
Hemin.....	1.0	mg
DI Water.....	880	ml

Combine ingredients and autoclave at 121 °C for 15 minutes.
Aseptically add 20 ml of buffer solution (below).
Add 100 ml of heat-inactivated fetal calf serum

Buffer Solution

KH ₂ PO ₄	18.1	g
NaHPO ₄	25.0	g
DI Water.....	1000	ml

Autoclave at 121°C for 15 minutes.