

Preparation of medium B:

Add to 1 litre of sea water (33 PSU) filtered through 0,22 µm:

- 0,1 mL stock Trace elements with EDTA

Autoclave for 15 minutes, let cool for at least one day

After cooling add sterile per litre:

- 0,9 mL stock Trace elements with EDTA

- 1 mL stock Nutrients

- 0,5 mL Vitamine stock

Preparation of stock solutions:

Trace elements with EDTA	final stock	stock 1	stock 2
	g/100 mL	g/25 mL	g/25 mL
Na ₂ EDTA	0,372		
Fe- II-citrate	0,131		
MnCl ₂ ×4H ₂ O		0,495	
ZnSO ₄ ×7H ₂ O		0,072	
Na ₂ MoO ₄ ×2H ₂ O		0,060	
CoCl ₂ ×6H ₂ O		0,030	
CuSO ₄ ×5H ₂ O			0,062
H ₂ SeO ₃			0,032
NaVO ₃			0,030
NiSO ₄ ×6H ₂ O			0,066

Prepare individual solutions for each trace element of the series of stocks 1 and 2.

Add 1 mL of each stock 1 and 0,1 mL of each stock 2 in 100 mL of final stock (working stock). Filtrate final stock (0,2 µm sterile, non-pyrogenic syringe filter) and store at 4 °C.

Filtered stocks 1 and stocks 2 can be stored at 4 °C.

Nutrients	stock
	g/50 mL
Na NO ₃	3,75
Na ₂ glyceroPO ₄	0,153
NaH ₂ PO ₄	0,25

Filtrate stock (0,2µm sterile non-pyrogenic syringe filter) and keep at 4 °C.

Vitamins	final stock	stock 1	stock 2
	mg/100 mL	mg/50 mL	mg/50 mL
Thiamine-HCl	20 mg		
Biotin		1,04	
Vitamin B ₁₂			11,1

In 100 mL of final stock (vitamin working stock) add 0,5 mL of stock 1 and stock 2.

Filtrate final stock (0,2 µm sterile, non-pyrogenic syringe filter) and keep at -20 °C.

Filtered stock 1 and stock 2 can be stored at -20 °C.