

Preparation of medium BT:

Add to 1 litre of sea water (35 PSU) filtered through 0,22 µm:

- 0,1 mL stock Trace elements with EDTA
- 1 ml stock buffer TRIS pH 7,2

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

After cooling add sterile per litre:

- 0,4 mL stock Trace elements with EDTA
- 0.5 ml nutrient stock 1
- 0.5 ml nutrient stock 2
- 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	3,72		
Fe- II-citrate	0,131		
MnCl ₂ x4H ₂ O		0,495	
ZnSO ₄ x7H ₂ O		0,072	
Na ₂ MoO ₄ x2H ₂ O		0,060	
CoCl ₂ x6H ₂ O		0,030	
CuSO ₄ x5H ₂ O			0,062
H ₂ SeO ₃			0,032
NaVO ₃			0,030
NiSO ₄ x6H 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 (0,2µm sterile, non-pyrogenic syringe filter) and keep at 4 °C.

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

Nutrients	stock 1	stock 2
	g/50 ml	g/50 ml
Na NO ₃	3,75	
Na ₂ glyceroPO ₄	0,153	
NaH ₂ PO ₄	0,25	
NH ₄ Cl		0,134

Filtrate nutrient stock 1 and stock 2 (0,2µm sterile non-pyrogenic syringe filter) and keep separately at 4 °C.

TRIS buffer	stock
	g/50 ml
TRIS-HCl*	7,09
TRIS base	0,605

*SIGMA. Filtrate stock (0,2 µm sterile non-pyrogenic syringe filter) and keep at 4 °C. Alternatively the solution can be made using TRIS base adjusting the pH with HCl.

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
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In 100 ml of final stock (vitamin working stock) add 0,5 ml of stock 1 and stock 2. Filtrate final stock (0,2um sterile, non-pyrogenic syringe filter) and keep at -20 °C. Filtered stock 1 and stock 2 can be stored at -20 °C.