

K Medium

Stocks

- | | per 100ml |
|--|------------------|
| (1) NaNO ₃ | 7.5 g |
| (2) NH ₄ Cl | 0.267 g |
| (3) Na ₂ b-glycerophosphate | 0.216 g |
| (4) H ₂ SeO ₃ | 0.0129 g |
| (5) Tris-base (7.2pH) | 12.11 g |
- (First add to 50ml of DIW and pH. Then slowly top up to 100ml with DIW ensuring to check pH remains at 7.2.)

- (6) Trace elements

There are 7 chemicals. Prepare Primary stock solutions first. Add first three chemicals separately in **100ml/dH₂O**, allowing each to completely dissolve before adding the primary stocks in order:

	Chemical	Primary stock solutions 10ml dH₂O	Amount/Volume for 100 ml working stock solution
1	Na ₂ EDTA	-	4.36 g
2	FeCl ₃ ·6H ₂ O	-	3.15 g
3	MnCl ₂ ·4H ₂ O	-	0.18 g
4	CuSO ₄ ·5H ₂ O	0.025 g	1 ml
5	ZnSO ₄ ·7H ₂ O	0.22 g	1 ml
6	CoCl ₂ ·6H ₂ O	0.1 g	1 ml
7	Na ₂ MoO ₄ ·2H ₂ O	0.063 g	1 ml

- (7) Vitamin mix: First make primary stocks of Cyanocobalamin and Biotin.

	per 100 ml
Cyanocobalamin (Vitamin B ₁₂)	0.1 g
Biotin	0.1 g

Dispense any excess primary stocks into 1 ml aliquots and freeze.

	per 200 ml
For final vitamin mix stock solution:	
Thiamine HCl (Vitamin B ₁)	0.1 g
Cyanocobalamin (Vitamin B ₁₂)	1 ml
Biotin	1 ml

Medium

	per litre
Stock solutions 1 - 3	1.0 ml each
Stock solution 4	0.01 ml
Stock solution 5 Tris-base (7.2pH)	1.0 ml
Stock solution 6 (Trace element)	0.1 ml
Stock solution 7 (Vitamin mix)	1.0 ml

Make up to 1 litre with filtered natural seawater. Adjust pH to **8.0** with 1M NaOH or 1M HCl. Autoclave at 15 psi for 15 minutes.

Reference K-medium (after Keller and Selvin, 1987) – adapted for CCAP

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