UCM – *Ulva* Culture Medium

Before preparing this media you must read and sign the UCM Media Prep SSW/RA

Stocks		**per litre**
(1)	$NaH_2PO_4.H_2O$	0.7 g
	$NaHCO_3$	8.8 g
	Tris	10.0 g
	Filter sterile.	
(2)	KBr	7.84 g
	KCl	54.2 g
	$SrCl_2.6H_2O$	1.95 g
	Filter sterile.	
(3)	Trace elements (x10 concentration):	**per litre**
	Na_2EDTA	6.684 g
	H_3BO_3	11.4 g
	$FeSO_4.7H_2O$	1.99 g
	$CuSO_4.5H_2O$	0.039 g
	$Na_2MoO_4.2H_2O$	0.126 g
	$MnCl_2.4H_2O$	0.36 g
	$ZnSO_4.7H_2O$	0.44 g
	$Co(NO_3)_2.6H_2O$	0.033 g
	NH_4VO_3	0.023 g
	KI	0.039 g
	$Na_2SeO_3.5H_2O$	0.00263g
	As_2O_3	0.000093 g
	$Na_2WO_4.2H_2O$	0.000066 g
	TeO_2	0.000034 g

For ease of measuring, first prepare primary trace element stock solutions.

Primary stock 1		**per 100 ml**
	$Na_2SeO_3.5H_2O$	2.63 g
Primary stock 2		
	NaOH	4.0 g
	As_2O_3	0.093 g
Primary stock 3		
	$Na_2WO_4.2H_2O$	0.066 g
Primary stock 4		
	NaOH	4.0 g
	TeO_2	0.034 g

Filter sterile and freeze in 1 ml aliquots.

For final trace element stock, add all chemicals except those included in the primary trace element stocks, then add 0.1 ml per litre of each primary trace element stock.

Mixture should be pale opaque yellow in colour and will be strongly acidic. Adjust pH to 8.0 using 5M NaOH. As the pH changes, the mixture should turn transparent and deep amber in colour. Filter sterile.

(4)

Vitamin stock	**per litre**
Inositol	2.0 g
Thymine	1.6 g
Choline chloride	0.72 g
Orotic acid	0.52 g
Thiamine HCL	0.4 g
Niacin	0.2 g
Ca-panthothenate	0.2 g
Pyridoxine.HCL	0.08 g
Putrescine	0.08 g
p-aminobenzoic acid	0.02 g
Biotin	0.01 g
Riboflavin	0.01 g
Folic acid	0.005 g
Folinic acid (citrovorum)	0.0004 g
Cyanocobalamin	0.0001 g
Pyridoxamine.2HCl	0.00004 g

For ease of measuring, first prepare primary vitamin stock solution.

Primary vitamin stock	**per 100 ml**
Folinic acid (citrovorum)	0.04 g
Cyanocobalamin	0.01 g
Pyridoxamine.2HCl	0.004 g

Filter sterile and freeze in 1 ml aliquots.

For final vitamin stock, add all vitamins except those included in the primary vitamin stock, then add 1 ml per litre of the primary vitamin stock.

Filter sterile.

Medium	**per litre**
NaCl	19.14 g
$Na_2SO_4.10H_2O$	7.28 g
$MgCl_2.6H_2O$	8.68 g
$CaCl_2.6H_2O$	1.24 g
$NaNO_3$	0.085g
$(NH_4)_2SO_4$	0.0066 g
Stock solution 1*	10 ml
Stock solution 2*	10 ml
Stock solution 3 (Trace elements)*	1 ml
Stock solution 4 (Vitamins)*	1 ml

Make up to 978 ml with deionised water and adjust pH to 8.1 with 1M NaOH or 1M HCl. Autoclave at 15 psi for 15 minutes.

After medium has cooled, add sterilised stocks 1-4.

Reference

Stratmann, J., Paputsoglu, G. and Oertel, W. (1996), DIFFERENTIATION OF *ULVA MUTABILIS* (CHLOROPHYTA) GAMETANGIA AND GAMETE RELEASE ARE CONTROLLED BY EXTRACELLULAR INHIBITORS. Journal of Phycology, 32: 1009-1021. – adapted for CCAP

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