Worden Lab
Prepared by: Marie Cuvelier & Melinda Simmons
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Keller (K) medium in artificial seawater

(Keller et al., 1987)

This recipe describes how to make K media in artificial seawater

Artificial seawater can also be used to make other types of media. Similarly, the K media can be made using "real" seawater. We use this media for growing some Prasinophytae, Mamiellales strains; however, note that not all seem to tolerate ASW.

General notes:

- We try as much as possible to work in non-"dead" areas, i.e. to avoid equipment or spaces where fixatives have been used.
- All the chemicals should be handled with plastic spatulas (we use sterile disposable spatulas) and weighed in plastic trays (or on weighing wax paper). Do not use metal spatulas.
- The ASW and media are prepared and autoclaved in acid cleaned polycarbonate bottles that have never been used with toxic chemicals.
- When autoclaving the ASW, do not fill more than half of the bottle with liquid to avoid spillage in autoclave.
- Once the ASW is autoclaved, it is handled and cooled to room temperature in the laminar flow hood to avoid contamination.

Artificial Seawater (ASW) - final volume = 1L

- 24.55 g of NaCl (Sigma S3014)
- 0.75 g of KCl (Sigma P9333)
- 4.07 g $MgCl_2.6H_2O$ (Sigma M2670)
- 1.47 g $CaCl_2.2H_2O$ (Sigma C5080)
- 6.04 g $MgSO_4.7H_2O$ (Sigma M5921)
- 0.21 g $NaHCO_3$ (Sigma S6297) (added last to adjust pH)

Bring to 1 L with 18.2 MΩ H_2O, e.g. MilliQ or Nanopure.
(If you only want ASW: autoclave this solution and cool to room temperature, otherwise proceed to K medium components)

K Medium – final volume = 1L

- Add the following K medium stock solutions (see additional notes on K medium stock solutions for stock solution concentrations and final concentrations) to the 1L of ASW (note the final concentrations here will be slightly off, since you will end up with 1.0055 L of solution; if you want to avoid this you need to start with slightly less ASW and then fill to 1L after adding the K components):

- 1 mL of $NaNO_3$ (8.82 X 10^{-4} M)/ NH_4Cl (5.00 X 10^{-5} M): use 1 mL of the solution containing both sources of N (NO_3 and NH_4) **or** 1 mL of **<u>each</u>** solution (1 mL of $NaNO_3$ **<u>and</u>** 1 mL of NH_4Cl) if they are separate solutions.
- 1 mL of Na_2 beta-glycerophosphate $6H_2O$ (1.00X10^{-5} M)
- 1 mL of H_2SeO_3 (1.00X10^{-8} M)
- 1 mL of Tris-base (1.00X10^{-3} M)
- 1 mL of K trace metal mix
- 0.5 mL of F/2 vitamins (kept at 4ºC or -20ºC, see notes below)
- Autoclave in acid cleaned polycarbonate bottles and cool to room temperature. Note that some groups do not autoclave vitamins, but rather add them afterwards. Because many of our cultures are axenic, we autoclave the entire media solution (with vitamins).
- Check pH and adjust for a final pH between 8.12 and 8.25:
- To avoid contamination, only use the pH meter probe in an aliquot (usually few mL) removed from the media bottle. In our lab, the pH of the medium before adjustment is always too basic (usually between 8.4 and 8.9).
- Adjust pH by adding filtered sterilized (0.2 µm) 4M NaOH or 10% HCl directly into the medium. Repeat these steps until the pH is between 8.1 and 8.2. (Both NaOH and HCl should be made with sterile MilliQ or Nanopure and handled in the hood. We typically prepare the solutions in 50 mL conical tubes.)

Notes on K medium stock solutions (see next page for components)

- All medium solutions are made in acid cleaned glass bottles and are autoclaved (i.e. we autoclave all stock solutions except for the vitamin stock, see below).
- After autoclaving they should be handled in the laminar flow hood using sterile pipettes to avoid contamination. The stocks are stored at room temperature in the dark except for vitamins (see notes below).
- The following tables describe the concentration of each solution and the final molar concentration once the appropriate amount has been added to the medium. These tables are a slight modification from the tables that are found on the CCMP (The Provasoli-Guillard National Center for Culture of Marine Phytoplankton) website: http://ccmp.bigelow.org/

K medium stock solutions

(modified from Keller et al., 1987)

Compound	Stock solution concentration (make in 18.2 MΩ H_2O)	Amount to add to ASW (quantities are for a final volume of 1L of media)	Final molar concentration in KASW media
$NaNO_3$	75 g / L	1 mL	8.82×10^{-4} M
NH_4Cl	2.67 g / L	1 mL	5.01×10^{-5} M
Na_2 beta-glycerophosphate $6H_2O$	2.16 g / L	1 mL	1.00×10^{-5} M
H_2SeO_3	1.29 mg / L	1 mL	1.00×10^{-8} M
Tris-HCl (pH=7.2)*	1 M	1 mL	1.00×10^{-3} M
K trace metals	see recipe below	1 mL	-
F/2 vitamins	see recipe below	0.5 mL	-

* The CCMP website recommends: Compound = Tris-base (pH=7.2); Stock solution concentration = 121.1 g / L. We use the Trizma® hydrochloride buffer solution pH 7.2, 1 M (Sigma T2069).

K trace metals stock solution

(from CCMP, 2007)

Compound	Stock solution concentration (make in 18.2 MΩ H_2O)	Amount to add to 18.2 MΩ H_2O** (quantities are for a final volume of 1L of trace metals stock solution)	Final molar concentration in KASW media
$Na_2EDTA \cdot 2H_2O$*	-	41.60 g	1.12×10^{-4} M
$FeCl_3 \cdot 6H_2O$	-	3.15 g	1.17×10^{-5} M
$Na_2MoO_4 \cdot 2H_2O$	6.3 g / L	1 mL	2.60×10^{-8} M
$ZnSO_4 \cdot 7H_2O$	22.0 g / L	1 mL	7.65×10^{-8} M
$CoCl_2 \cdot 6H_2O$	10.0 g / L	1 mL	4.20×10^{-8} M
$MnCl_2 \cdot 4H_2O$	180.0 g / L	1 mL	9.10×10^{-7} M
$CuSO_4 \cdot 5H_2O$	9.8 g / L	1 mL	3.92×10^{-8} M

***** Make the EDTA first then add the Iron(III) chloride hexahydrate, followed by the rest of the metals. The solution is then autoclaved and stored at room temperature or 4ºC, in the dark.
******We use 18.2 MΩ H_2O, CCMP suggests deionized H_2O
We first make primary stocks (using pre-autoclaved 18.2 MΩ H_2O) that are filter sterilized into 15 mL sterile conical tubes and frozen at -20°C. These are thawed (when a new working stock is needed), diluted to make a single working stock and kept at room temperature in the dark.

F/2 vitamins stock solution

(Guillard & Ryther 1962, Guillard, 1975)

- F/2 vitamin solution is made in acid cleaned glass bottles and sterilized. It should be handled in the laminar flow hood and with sterile pipettes to avoid bacterial contamination.
- The following table describes the concentration of F/2 vitamin solution and the final molar concentration once the appropriate amount has been added to the media. This table is a slight modification from the table found on the CCMP (The Provasoli-Guillard National Center for Culture of Marine Phytoplankton) website: http://ccmp.bigelow.org/

Compound	Stock solution concentration (make in 18.2 MΩ H_2O)	Amount to add to pre-autoclaved 18.2 MΩ H_2O (quantities are for a final volume of 1L of vitamin stock solution)	Final molar concentration in KASW media
Vitamin B_{12} (cyanocobalamin)	1 g / L	1 mL	3.69×10^{-10} M
Biotin	0.1 g / L	10 mL	2.05×10^{-9} M
Thiamine . HCl		200 mg	2.96×10^{-7} M

We first make primary B_{12} and Biotin stocks (using pre-autoclaved 18.2 MΩ H_2O) that are filter sterilized into 15 mL sterile conical tubes and frozen at -20°C. These are thawed (when a new working stock is needed), diluted to make a single working stock (with the Thiamine added) and filter sterilized into ~ single use aliquots (in sterile 1.5 mL eppendorf tubes) that are stored at -20°C. Once in use these aliquots are kept at 4ºC for about 2 weeks; this allows us to avoid multiple freeze thaw events.

Ordering info for chemicals used

REAGENT	VENDOR	CATALOG NUMBER
$NaNO_3$	JTBaker	3770-01
NH_4Cl	Sigma	A9434
Na_2 beta-glycerophosphate $6H_2O$	Fluka	50020
H_2SeO_3	Sigma Aldrich	211176
Tris-HCl (pH=7.2)*	Sigma	T2069
$Na_2EDTA \cdot 2H_2O$*	Sigma	E5134
$FeCl_3 \cdot 6H_2O$	JTBaker	2000-01
$Na_2MoO_4 \cdot 2H_2O$	EMScience	SX0650-2
$ZnSO_4 \cdot 7H_2O$	JTBaker	4384-01
$CoCl_2 \cdot 6H_2O$	Mallinckrodt	4532
$MnCl_2 \cdot 4H_2O$	JTBaker	2540-04
$CuSO_4 \cdot 5H_2O$	JTBaker	1843-01
Vitamin B_{12} (cyanocobalamin)	Sigma	V6629
Biotin	Sigma	B4639
Thiamine . HCl	Sigma	T3902

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