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Keller (K) Optimized Medium for Culturing Microalgae

Keller (K) Medium in Artificial Sea Water (ASW) for Culturing Microalgae (Ostreococcus tauri)

Roscoff Culture Collection[1], Lynn Doran[2], Steven J Burgess[3]

[1]CNRS-Sorbonne Université, Station Biologique, Place G. Tessier 29680 Roscoff FRANCE;

[2]Realizing Increased Photosynthetic Efficiency (RIPE); [3]University of Illinois at Urbana-Champaign

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Burgess Lab UIUC

Steven Burgess
University of Illinois at Urbana-Champaign

This protocol requires modifications for diatoms.

Medium to grow most phytoplankton species in small eukaryotes (Mamiellophyceae, Pelagophyceae etc...).
Optimized for Ostreococcus tauri. Keller Optimized Medium was developed by Smallwood et al. (https://doi.org/10.1101/291211) which includes additions of 17 mM sodium nitrate and 0.37 mM NaH2PO4

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protocol

Keller, M.D., Selvin, R.C., Claus, W. & Guillard, R.R.L. 1987. Media for the culture of oceanic ultraphytoplankton. J. Phycol. 23:633–8.

Keller (K) Medium in Artificial Sea Water (ASW) for Culturing Microalgae (Ostreococcus tauri), Lynn Doran

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K medium, Keller medium, Artificial Sea Water, ASW, Culture, Culturing, Algae, Ostreococcus, O. tauri

protocol ,

Lynn Doran

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Not all algae cultures tolerate artificial sea water and many culture stock centers will not guarantee culture growth in artificial seawater based media. We have had success with O. tauri using K medium made in artificial sea water.

Volumes of stock solutions and K medium are given for AgSynBio Lab's average consumption of ASW + K Medium. Volumes may need to be scaled up or down depending on amount of algae being cultured weekly.

Reference

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26-60. In Smith W.L. and Chanley M.H (Eds.) Culture of Marine Invertebrate Animals. Plenum Press, New York, USA.

Keller, M.D. and Guillard, R.R.L. 1985. Factors significant to marine diatom culture. pp. 113-6. In Anderson, D.M., White, A.W. and Baden, D.G. (eds.) Toxic Dinoflagellates. Elsevier, New York.

Keller, M.D., Selvin, R.C., Claus, W. & Guillard, R.R.L. 1987. Media for the culture of oceanic ultraphytoplankton. J. Phycol. 23:633–8.

Sambrook, J. and Russell, D.W. 2001. Molecular Cloning: A Laboratory Manual. 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

van Ooijen, G., Knox, K., Kis, K., Bouget, F. Y., & Millar, A. J. (2012). Genomic transformation of the picoeukaryote Ostreococcus tauri.Journal of visualized experiments : JoVE, (65), e4074. https://doi.org/10.3791/4074

Additional Information

K and K+Si medium, Roscoff Culture Collection

K Medium, Bigelow National Center for Microalgae and Microbiota

Keller (K) Medium in Artificial Seawater, Worden Lab at Monterey Bay Aquarium Research Institute

Making Media by Australian Algae National Culture Collection

Reagents

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A	B
Chemical	**Manufacturer and Product ID**
18.2 MΩ water	Milli-Q or Nanopure Water Filtration System
Biotin (Vit H)	Sigma B4639
Vitamin B12	Sigma V6629
CuSO4• 5H2O	Sigma C8027
MnCl2 • 4H2O	Sigma M3634
CoCl2• 6H2O	Sigma C8661
ZnSO4 • 7H2O	Sigma Z0251
Na2MoO4 • 2H2O	Sigma M1651
HCl 37%	Sigma 320331
NaOH	Sigma S8045
Tris-Base	Sigma T6066
H2SeO3	Sigma 211176
β-Glycerophosphate disodium salt hydrate xhydrate	Sigma G9422
NH4Cl	Sigma A9434
NaNO3	Sigma S5022
Thiamine • HCl (B1)	Acros Organics 148990100
FeCl3 • 6H2O	Sigma 236489
Na2EDTA • 2H2O	Acros Organics 147855000
NaCl	Sigma S7653
KCl	Sigma P5405
MgCl2.6H2O	Fisher BP214
CaCl2.2H2O	Phytotech Labs C135
MgSO4.7H2O	Sigma 230391
NaHCO3	Sigma S6014

Reagent ordering information for Keller Medium in Artificial Sea Water

Equipment

- Media Storage Bottle, 1 L
- Media Storage Bottle, 100 mL
- Graduated cylinder, 1 L
- Graduated cylinder, 100 mL
- Graduated cylinder, 5 mL
- 50 mL Centrifuge Tubes, Sterile, Polypropylene, Globe Scientific (Fisher Scientific 22-010-066)
- 15 mL Centrifuge Tubes, Sterile, Polypropylene, Corning (Fisher Scientific 05-539-5)
- Microcentrifuge tube, 0.5 mL
- Pipette, 1-10 ul
- Pipette, 100 ul
- Pipette, 1000 ul
- Pipette tips, 10 ul

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- Pipette tips, 100 ul
- Pipette tips, 1000 ul
- Weigh paper, 4" x 4", Fisherbrand (Fisher Scientific 09-898-12B)
- Spatula, stainless (Fisher Scientific 14-375-20) or scoopula, stainless, 6" (Fisher Scientific 14-357Q)
- Microanalytical balance
- Balance, Analytical, Mettler-Toledo AB104-S
- 0.22 µM filters disposal and reusable contraption
- Autoclave
- Water bath, alternatively hot plate with a beaker of water
- pH Meter
- Chemical fume hood
- Laminar Flow Hood, NuAire Class II, Type A2

- Autoclaving has a high burn risk due to pressure, heat, and steam. Review all safety protocols and receive proper training before operating an autoclave.

- This protocol uses both chemical fume hoods and biological safety cabinets (laminar flow hoods). Understand the difference and how to safely and appropriately use both before performing the protocol.

- Many of the chemical components of the K Medium pose serious health risks. Please read all manufacturer safety data sheets before handling. UIUC personnel performing this protocol should be current on "Laboratory Safety", "Chemical Safety- An Introduction", and "Chemical Spills" Division of Research Safety training modules before performing this protocol.

- Many of the stock chemicals contain concentrations of heavy metals that may be toxic to the environment. Dispose of properly.

A	B	C	D
Chemical	**Hazard Rating**	**Safety Advice**	**Storage**
Biotin (Vit H)	Non-hazardous		Recommended dry, 2-8C. Non-combustible solid.
B12	Non-hazardous		Hygroscopic, light sensitive. Recommended storage 2-8C. Non-combustible solid
f2 vitamin	Non-hazardous		Dark, Freeze.
Thiamine-HCL	Eye irritation, forms combustible dust	Wear PPE, dispose gloves, wash face, hands, exposed skin.	Hygroscopic, light sensitive. Non-combustible solid

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$CuSO_4 \cdot 5H_2O$	Acute toxicity, Oral (Category 4), H302 Serious eye damage (Category 1), H318 Skin irritant	Wear PPE, dispose gloves, wash face, hands, exposed skin.	Air sensitive. Handle and store under inert gas. Hygroscopic Combustible Solids
$MnCl_2 \cdot 4H_2O$	Acute toxicity, Oral (Category 3) Serious eye damage (Category 1) Specific target organ toxicity - repeated exposure (Category 2)	Wear PPE, dispose gloves, wash face, hands, exposed skin. Weigh in fume hood.	Store locked up Non-combustible acute toxic
$CoCl_2 \cdot 6H_2O$	Acute toxicity, Oral (Category 4) Respiratory sensitisation (Category 1) Skin sensitisation (Category 1) Germ cell mutagenicity (Category 2) Carcinogenicity (Category 1B) Reproductive toxicity (Category 1B)	Wear PPE, dispose gloves, wash face, hands, exposed skin. Only use solid chemical in fume hood. Clean fume hood with 70% EtOH after use. Wash lab coat after use. Do not use if pregnant.	Store locked up Non-combustible acute toxic
$ZnSO_4 \cdot 7H_2O$	Acute toxicity, Oral (Category 4), H302 Serious eye damage (Category 1), H318	Wear PPE, dispose gloves, wash face, hands, exposed skin. Weigh in fume hood. Clean up any residual solids immediately.	hygroscopic combustible solid
$Na_2MoO_4 \cdot 2H_2O$	Irritant, avoid prolonged exposure	Clean up any residual solids immediately. Wear PPE.	combustible solid
Tris-base	Non-hazardous		hygroscopic Non-combustible solid
H_2SeO_3	Acute toxicity, Oral (Category 3), H301 Acute toxicity, Inhalation (Category 3), H331 Specific target organ toxicity - repeated exposure (Category 2), H373	Wear PPE, dispose gloves, wash face, hands, exposed skin. Weigh in fume hood.	hygroscopic Non-combustible toxic Locked up
β-Glycerophosphate disodium salt hydrate xhydrate	Non-hazardous		Non-combustible solid

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NH4Cl	Acute toxicity, Oral (Category 4), H302 Eye irritation (Category 2A), H319	Wear PPE, dispose gloves, wash face, hands, exposed skin.	Hygroscopic Non-combustible solid
NaNO3	Oxidizing solids (Category 3), H272 Eye irritation (Category 2A), H319	Wear PPE, dispose gloves, wash face, hands, exposed skin. KEEP AWAY FROM HEAT, FIRE, FLAME.	Strong Oxidizer
FeCl3 • 6H2O	Corrosive to Metals (Category 1), H290 Acute toxicity, Oral (Category 4), H302 Skin irritation (Category 2), H315 Serious eye damage (Category 1), H318	Wear PPE, dispose gloves, wash face, hands, exposed skin. Weigh in fume hood. Wash lab coat after use.	Store under inert gas. Hygroscopic. Non-combustible, corrosive hazardous materials
Na2EDTA • 2H2O	Eye irritation (Category 2A), H319	Wear PPE, dispose gloves, wash face, hands, exposed skin.	Non-combustible solid
NaCl	Non-hazardous		Non-combustible solid
KCl	Non-hazardous		Non-combustible solid
MgCl2.6H2O	Non-hazardous		Non-combustible solid
CaCl2.2H2O	Eye irritation (Category 2A), H319	Wear PPE, dispose gloves, wash face, hands, exposed skin.	Non-combustible solid
MgSO4.7H2O	Non-hazardous		Non-combustible solid
NaHCO3	Non-hazardous		Non-combustible solid

Safety Information for K Medium Chemical Components

:

> This protocol requires modifications for diatoms.

Please refer to the Roscoff Culture Center general recommendations to grow cultures.

This protocol makes 1 L of Keller Medium in Artificial Sea Water and the minimum

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possible amount of each working and stock solution. To automatically adjust weights and volumes for a different amount of ASW in K Medium or the component working and stock solutions use the following Batch Size Workbook:

Batch Size Workbook_ ASW + K Medium.xlsx

*Protocols.io steps sections are color coded to match the color coding for the reagent prep in the Batch Size
Workbook.

It is best practice to keep a log book of all information used to make K Medium in Artificial Sea Water and the stock solutions. In the event that there is an issue with culture growth, it will be easier to find the problem if this data is recorded.

AgSynBio ASW + K Medium Prep Log Book.xlsx **(Log in required)**

Example Template for ASW + K Medium Prep Log Book.xlsx

Prepare Artificial Sea Water (ASW)

1 ⚠

Prepare artificial sea water (ASW) (**1 L**).

Please note not all algae cultures tolerate artificial sea water and many culture stock centers will not guarantee culture growth in artificial seawater based media.

To prepare natural seawater per the Roscoff Center:

- Collect seawater (salinity ca. 33‰);
- **Leave the seawater (SW) to age for 2 months at room temperature in the darkness**;
  Note: *This"aging" step is critical because during that period bacteria present in the sea water will degrade many organic molecules that are probably detrimental to algae. The Roscoff Center's experince shows that medium made with 'fresh' seawater will in many cases prevent cells from growing.*
- Filter through 0.22µM filters (Millipore filter GSWP09000 plus Millipore prefilter AP1507500) and autoclave;
- Continue at step 2.

1.1 Fill a **1 L** media storage bottle two-thirds full with 18.2 MΩ water (Milli-Q or Nanopure).

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18.2 MΩ water (Milli-Q or Nanopure) is not sterile unless autoclaved.

1.2 Weigh the following chemicals using a spatula and weigh paper. Add them to the media storage bottle.

A	B
Chemical	**Amount**
Sodium chloride (NaCl)	24.55 g
Potassium chloride (KCl)	0.75 g
Magnesium chloride hexahydrate (MgCl2.6H2O)	4.07 g
Calcium chloride dihydrate (CaCl2.2H2O)	1.47 g
Magnesium sulfate heptahydrate (MgSO4.7H2O)	6.04 g

Chemical components and weights for 1 L of artificial sea water

Best practice is to use single use plastic utensils and weigh boats for weighing chemicals for ASW. To date, we have not had any issues with stainless steel, washable utensils (2/9/2021).

Best practice is to dedicate chemical supplies for ASW and K medium; keep chemicals separate from general lab supply. Trace amounts of cross contamination from other lab chemicals could kill algae cultures.

1.3 Swirl the bottle until all chemicals have dissolved. Weigh the following chemical using a spatula and weigh paper. Add them to the media storage bottle and swirl until dissolved.

A	B
Chemical	**Amount**
Sodium bicarbonate (NaHCO3)	0.21 g

Sodium bicarbonate weight for 1 L of artificial sea water

Sodium bicarbonate is added last after all other components have

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dissolved to adjust pH.

1.4 Bring to 1 L in a graduated cylinder with 18.2 MΩ H2O (Milli-Q or Nanopure). Return solution to media storage bottle.

1.5

Best practice would be to autoclave the artificial sea water before each use.

Final ASW + K Medium solution will be sterilized by autoclave (sterile) or 0.22 um membrane filter (some bacteria and viruses can pass). Because final solution will be sterilized, it is not necessary to sterilize the ASW only. If ASW will be stored for a long time between uses, it is advisable to sterilize to inhibit any biological growth from potential contaminants.

1.6 Label artificial sea water with analyst name, date, and the word sterile or non-sterile. Fill out the ASW + K Medium Prep Log Book.

Prepare Trace Metal Solutions

2 Prepare K Medium Trace Metal Working Stock Solution (7 mL).

2.1

Prepare each individual stock solution for Trace Metal Working Stock Solution.

NOTE: Each row of the table below is a separate stock solution and should be prepared in its own container. Do not mix the individual solutions at this time.

- Label a centrifuge tube (or microcentrifuge tube if applicable) for each individual stock solution. Include the name, concentration, date, and preparer's initials.
- Fill a centrifuge tube (or microcentrifuge tube if applicable) with 2/3 of the required total volume with 18.2 MΩ water.

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- Weigh the chemicals in a chemical fume hood on a micro analytical balance using a spatula and weigh paper. Add them to the appropriately labeled tube.
  NOTE: Weights below are in milligrams.
- Mix by shaking until chemical is completely dissolved.
- Pour solution into a graduated cylinder and bring to the total assigned volume with 18.2 MΩ water.
- Return to centrifuge tube. Shake well.
- Store for future use. Each individual working stock solution will make many batches of Trace Metal Working Solution.
- Fill out the ASW + K Medium Prep Log Book.
- Clean weighing utensils and graduated cylinder before preparing the next individual working stock solution.

A	B	C	D	E
Chemical	**Volume prepared (mL)**	**Weight of chemical (mg)**	**Concentration (g/L)**	**Batches of Trace Metal Solution (7 mL) that can be prepared from this volume**
$Na_2MoO_4 \cdot 2H_2O$	4	25.2	6.3	571
$ZnSO_4 \cdot 7H_2O$ (22.0 g/L)	1	22.0	22	143
$CoCl_2 \cdot 6H_2O$ (10.0 g/L)	2	20.0	10	286
$MnCl_2 \cdot 4H_2O$ (180.0 g/L)	0.2	36.0	180	29
$CuSO_4 \cdot 5H_2O$ (9.8 g/L)	2	19.6	9.8	286

Trace Metal stock solutions recipes

Many of the chemicals used to prepare K Medium Trace Metal Stock Solutions are acutely toxic. Review all safety information and applicable Safety Data Sheets before handling.

For long term storage, some labs have found in beneficial to store these solutions in the freezer if they will not be used in totality the day of preparation. AgSynBio lab currently stores them at room temperature with minimal issues. (2-22-2021)

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2.2 Prepare the trace metal working stock solution by adding **5 mL** 18.2 MΩ water to a new 15 mL centrifuge.

2.3 Weigh **0.29 g** EDTA Disodium Salt Dihydrate ($Na_2EDTA.2H_20$) on an analytical balance using a spatula and weigh paper.

2.4 ⚠

Add the EDTA Disodium Salt Dihydrate to the trace metal working stock solution. Cap and shake until completely dissolved.

EDTA must be added first and completely dissolved before the addition of the remaining chemicals in order to chelate the metal ions and prevent them from precipitating out of solution.

2.5 ☠

Weigh **20 mg** of hexahydrated ferric chloride ($FeCl_3.6H_20$) in a chemical fume hood on a microbalance using a spatula and weigh paper.

Hexahydrated ferric chloride ($FeCl_3.6H_20$) is acutely toxic. Review safety datasheet before handling.

2.6 Add the hexahydrated ferric chloride to the trace metal working stock solution. Cap and shake until completely dissolved.

If chemical won't dissolve after several minutes of vigorous shaking, indirectly heat solution in a water bath and continue intermittent shaking.

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2.7 Add the appropriate volume of the trace metal stock solutions to the trace metal working stock solution using a pipette.

A	B
Quantity	**Compound**
7 ul	Sodium Molybdate Dihydrate ($Na_2MoO_4.2H_2O$) Solution (6.3 g/L)
7 ul	Zinc Sulfate Heptahydrate ($ZnSO_4.7H_2O$) Solution (22.0 g/L)
7 ul	Cobalt Chloride Hexahydrate ($CoCl_2.6H_2O$) Solution (10.0 g/L)
7 ul	Manganese (II) chloride, tetrahydrate ($MnCl_2.4H_2O$) Solution (180.0 g/L)
7 ul	Copper(II) sulfate pentahydrate (Cu $SO_4.5H_2O$) Solution (4.9 g/L)

Stock solution volumes for 7 mL of K Medium Trace Metal Solution.

2.8 Bring final volume up to **7 mL** using 18.2 MΩ water. Shake to thoroughly mix all solutions. If any precipitate is visible, heat in a water bath.

The metals are reactive species. If disturbed they will form complexes and precipitate out. Precipitate can usually be re-solublized with indirect heating. If heating, doesn't cause re-solubilization than a pH adjustment may be necessary.

Autoclaving, freezing, photodegradation, changes in pH, and long term storage may cause precipitate to form.

2.9 Label trace metal working stock with analyst name and date. Fill out the ASW + K Medium Prep Log Book. Any trace metal working stock solution that will not be used the same day should be aliquoted and stored at **-20 °C** .

Prepare f/2 Vitamin Solutions

3 Prepare K Medium f/2 Vitamin Working Stock Solution (**100 mL**).

3.1 Prepare each individual stock solution for f/2 Vitamin Working Stock Solution.

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NOTE: Each row of the table below is a separate stock solution and should be prepared in its own container. Do not mix the individual solutions at this time.

- Label a centrifuge tube (or media storage bottle if applicable) for each individual stock solution. Include the name, concentration, date, and preparer's initials.
- Fill a centrifuge tube (or media storage bottle if applicable) with 2/3 of the required total volume with 18.2 MΩ water.
- Weigh the chemical on a micro analytical balance using a spatula and weigh paper. Add them to the appropriately labeled tube.
  NOTE: Weights below are in milligrams.
  NOTE: If the desired weight is the exact amount that comes in the reagent bottle, rinse the chemical directly from reagent bottle into the stock solution using 18.2 MΩ water.
- Mix by shaking until chemical is completely dissolved.
- Pour solution into a graduated cylinder and bring to the total assigned volume with 18.2 MΩ water.
- Return to centrifuge tube or media bottle. Shake well.
- Store for future use. Each individual working stock solution will make many batches of f/2 Vitamin Working Solution.
- Fill out the ASW + K Medium Prep Log Book.
- Clean weighing utensils and graduated cylinder before preparing the next individual working stock solution.
- Short term storage should be in the dark at **4 °C** , long term storage should be in the dark at **-20 °C** . Limit freeze/thaw of vitamin stock solutions in long term storage to three cycles.

Prepare each individual stock solution for f/2 Vitamin Working Stock Solution.

A	B	C	D	E
Chemical	**Total volume (mL)**	**Solid weight (mg)**	**D**	**Batches of f/2 Vitamin Working Solution (100 mL) made from this volume**
Biotin	100	10.0	0.1	100
Vitamin B12	20	20.0	1	200

f/2 stock solution recipes

3.2 Prepare the f/2 Vitamin working stock solution by adding **99 mL** 18.2 MΩ water to a new media storage bottle.

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3.3 Add the appropriate volume of the f/2 vitamin stock solutions to the f/2 Vitamin working stock solution using a pipette.

A	B
Quantity	**Compound**
100 ul	Vitamin B12 Solution (1 g/L)
1000 ul	Biotin Solution (0.1 g/L)

Stock solution volumes for 100 mL of f/2 Vitamin Working Stock Solution

3.4 Mix f/2 vitamin working stock solution by inverting several times.

3.5 Label f/2 vitamin working stock with analyst name and date. Fill out the ASW + K Medium Prep Log Book. Any f/2 vitamin working stock solution that will not be used the same day should be aliquoted and stored in the dark at **-20 °C** .

Limit freeze/thaw of f/2 vitamin working stock solutions to three cycles.

Prepare Keller Medium in ASW

4 Prepare Keller Medium in artificial seawater (**1000 mL**).

4.1 Prepare each individual stock solution for Keller Medium.

NOTE: Each row of the table below is a separate stock solution and should be prepared in its own container. Do not mix the individual solutions at this time.

- Label a centrifuge tube (or media storage bottle if applicable) for each individual stock solution. Include the name, concentration, date, and preparer's initials.
- Fill a centrifuge tube (or media storage bottle if applicable) with 2/3 of the required total volume with 18.2 MΩ water.
- Weigh the chemical on a micro analytical balance using a spatula and weigh paper. Add them to the appropriately labeled tube.
  NOTE: Weights below are in milligrams.
- Mix by shaking until chemical is completely dissolved.

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- Pour solution into a graduated cylinder and bring to the total assigned volume with 18.2 MΩ water.
- Return to centrifuge tube or media bottle. Shake well.
- Store for future use. Each individual working stock solution will make many batches of Keller Medium.
- Fill out the ASW + K Medium Prep Log Book.
- Clean weighing utensils and graduated cylinder before preparing the next individual working stock solution.

Prepare each individual stock solution for Keller Medium according to the table below.

A	B	C	D	E
Chemical	**Total volume (mL)**	**Solid (mg)**	**Concentration in final solution (g/L)**	**Batches of Keller Medium (1 L) made from this volume**
NH4Cl	8	21.4	2.68	8
β-glycerophosphate	10	21.6	2.16	10
H2SeO3	1000	1.29	0.00129	1000
Tris-base(pH 7.2)	1	121.1	121.1	1

Keller Medium stock solution recipes

4.2 Prepare Keller Medium by measuring **993.5 mL** of artificial sea water into a graduated cylinder.

4.3 Add the appropriate volume of the Keller medium stock solutions, trace metal working stock solution, and the f/2 Vitamin working stock solution to the Keller Medium in the graduated cylinder using a pipette.

A	B
Quantity	**Stock Solution**
1 mL	NH4Cl (2.68 g/L)
1 mL	β-glycerophosphate (2.16 g/L)
1 mL	H2SeO3 (0.00129 g/L)
1 mL	Tris-base(pH 7.2) (121.1 g/L)
1 mL	K trace metal solution
0.5 mL	f/2 vitamin solution
1.44 g	NaNO3 (17 mM final)
1 mL	NaH2PO4 stock

Stock and working stock volumes for 1 L of Keller Medium in Artificial Seawater

Citation: Roscoff Culture Collection, Lynn Doran, Steven J Burgess Keller (K) Optimized Medium for Culturing Microalgae https://dx.doi.org/10.17504/protocols.io.36wgq76z3vk5/v1

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4.4 pH Keller Medium and adjust pH to 8.1-8.2 using 10% hydrochloric acid (HCl) and 4 M sodium hydroxide (NaOH).

To make 10% HCl:

- Measure **22 mL** of 18.2 MΩ water into a centrifuge tube.
- In a chemical fume hood, add **8 mL** of 37% concentracted hydrochloric acid. **Always ADD ACID.**
- Cap and shake until mixed.
- Label with chemical name, analyst initials, date, and concentration.
- Fill out the ASW + K Medium Prep Log Book.

To make 4 M NaOH:

- Measure **20 mL** of 18.2 MΩ water into a centrifuge tube.
- Weigh **4.8 g** of sodium hydroxide pellets onto weigh paper using a spatula.
- In a chemical fume hood, slowly add the sodium hydroxide to the water. **Exothermic reaction. Add pellets slowly and allow solution to cool to handling temperature before adding more pellets.**
- Cap and shake until mixed.
- Label with chemical name, analyst initials, date, and concentration.
- Fill out the ASW + K Medium Prep Log Book.

Strong acids and bases can cause burns.

4.5 Filter sterilize the Keller Medium in artificial seawater into a sterile media storage bottle using a 0.22 micron membrane filter.

Filter sterilization will not remove 100% of bacteria or viruses. If culture is axenic or is not a robust culture, add everything but the f/2 vitamin and trace metals solutions, autoclave, and filter sterilize the f/2 vitamin and trace metals solutions into the finished medium.

If zero tolerance for biological contamination is required, autoclave the complete keller medium. Some vitamin efficacy will be lost during autoclaving as f/2 vitamin solution is heat-labile and white precipitate may form due to the reactivity of the trace metals under heat and pressure.

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4.6 Label Keller Medium with analyst name, date, and the word sterile. Fill out the ASW + K Medium Prep Log Book.

4.7 Store Keller Medium in the dark at **4 °C** for up to 2 weeks. Only open Keller Medium in a sterile, laminar flow hood.

f/2 vitamin stability limits the shelf life for Keller Medium. B1 and B12 alone are shelf stable but when in formulation together can lose up to 20% efficacy in 5 days, unless frozen.

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