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|  | **Culture medium for marine diatoms** **f/2 + Si Medium** (Guillard, 1975\*)The standard marine diatom culture medium

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| **Component** | **Quantity** |
| Sterile-filtered natural seawater |  1000 mL |
| ***Major nutrients***NaNO3NaH2PO4.H2ONa2SiO3.9H2O***Traces***Na2.EDTAFeCl3.6H2OCuSO4.5H2OZnSO4.7H2OCoCl2.6H2OMnCl2.4H2ONa2MoO4.2H2O***Vitamins ( if considered necessary)****\*Absence of vitamins doesn’t seem to affect diatoms****.***Thiamin.HClBiotinB12  | 75 mg/L5 mg/L30 mg/L4.36 mg/L3.15 mg/L0.01 mg/L0.022 mg/L0.01 mg/L0.18 mg/L0.006 mg/L0.1 mg/L0.5 µg/L0.5 µg/L |

Concentrated stock solutions of the different nutrients should be made, for instance 1000X. Prepare a concentrated stock solution of each **major nutrient** in separate recipients. All **trace elements** can be mixed in a single recipient. This gives a total of 4 different stock solutions. We prepare 200 mL of 1000X concentrated stock solutions in standard 250 mL Cell Culture Flasks (polystyrene) with a standard screw cap from Greiner Bio-One. Add the appropriate amount of each stock solution to a bottle with some distilled water, and adjust to the desired volume with distilled water. For instance, making 1L of WC medium with 1000X concentrated stock solutions means adding 1 mL of each stock solution to some distilled water, and adjusting to 1L with additional distilled water. Autoclave 1 L bottles of medium for 15 min and 2 L bottles for 20 min.Keep the sterilized medium in a fridge. |
|  | Alternatives:* In case you prefer using **artificial sea water**, you might use commercially available Tropic Marin Pro-Reef Salt. From our experience, artificial seawater prepared with other salt from another source can negatively affect the health and growth of diatom cultures. Follow the recipe as described above but use Tropic Marin Pro-Reef Salt – 35 g/L of dH2O instead of natural seawater.
* **Sigma Aldrich** sells a frozen 50X concentrated stock solution of all nutrients together, without or already with silica - **Guillard’s (F/2) Marine Water Enrichment Solution.**

We have good experience with this stock solution, and you can order this if you don’t want to make your own stock solution. You can add the nutrients before or after (aseptic!) autoclaving. Autoclaving will destroy the vitamins, but we don’t see any negative effect of this on our diatom cultures.

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| **Useful information from ‘Algal-culturing techniques’ by Robert A. Anderson, 2005:** It is recommended to use Standard Cell Culture Flasks (polystyrene) from Greiner Bio-One because:* + Phosphate stock solutions should never be stored in the polyethylene bottles since phosphate ions are strongly adsorbed onto polyethylene (Hassenteufel et al. 1963)
	+ Silicate stock solutions should be stored in non-vitreous material because of dissolution of silicic acid from glass vessels.
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