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|  | **Culture medium for freshwater diatoms**  **WC Medium**  (Guillard & Lorenzen, 1972. J Phycol 8:10-14)  The standard culture medium for freshwater diatoms from habitats with neutral to high pH   |  |  | | --- | --- | | **Component** | **Quantity** | | Distilled water | 1000 mL | | ***Major nutrients***  Ca Cl2.2H2O  MgSO4.7H2O  NaHCO3  K2HPO4  NaNO3  Na2SiO3.9H2O  ***Traces***  Na2.EDTA  FeCl3.6H2O  CuSO4.5H2O  ZnSO4.7H2O  CoCl2.6H2O  MnCl2.4H2O  Na2MoO4.2H2O  H3BO3  ***Vitamins (if considered necessary)***  *\*Absence of vitamins doesn’t seem to affect diatoms****.***  Thiamin.HCl  Biotin  B12 | 36.76 mg/L  36.97 mg/L  12.60 mg/L  8.71 mg/L  85.01 mg/L  28.42 mg/L  4.36 mg/L  3.15 mg/L  0.01 mg/L  0.022 mg/L  0.01 mg/L  0.18 mg/L  0.006 mg/L  1.0 mg/L  0.1 mg/L  0.5 µg/L  0.5 µg/L | |

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|  | 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 7 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.  In the original description by Guillard & Lorenzen (1972), **vitamins** are added after autoclaving. However, we stopped doing this many years ago, as it doesn’t seem to affect the health and growth of diatoms.  The **pH** of the WC culture medium should be between 7 and 8 after autoclaving. Adjust the pH with either 1 N NaOH or 1 N HCl. We have good experience however with using WC medium without pH check or adjustment.  Keep the sterilized medium in a fridge.   |  | | --- | | **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 polyethylene bottles since phosphate is 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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|  | **PM Medium**  Culture medium for freshwater diatoms from acidic habitats.   |  |  |  | | --- | --- | --- | | **Component** | **Quantity** | **Equivalent**  **to WC medium** | | Distilled water | 1000 mL | 1000 mL | | ***Major nutrients***  Ca Cl2.2H2O  MgSO4.7H2O  NaHCO3  K2HPO4  NaNO3  Na2SiO3.9H2O  ***Traces***  Na2.EDTA  FeCl3.6H2O  CuSO4.5H2O  ZnSO4.7H2O  CoCl2.6H2O  MnCl2.4H2O  Na2MoO4.2H2O  H3BO3  ***Vitamins (if considered necessary)***  *\*Absence of vitamins doesn’t seem to affect diatoms****.***  Thiamin.HCl  Biotin  B12 | 0.37 mg/L  3.7 mg/L  3.15 mg/L  2.9 mg/L  56.7 mg/L  14.21 mg/L  2.18 mg/L  1.58 mg/L  0.005 mg/L  0.011 mg/L  0.005 mg/L  0.09 mg/L  0.003mg/L  0.5 mg/L  0.1 mg/L  0.5 µg/L  0.5 µg/L | 1/100 WC  1/10 WC  1/4 WC  1/3 WC  2/3 WC  1/2 WC  1/2 WC  1/2 WC  1/2 WC  1/2 WC  1/2 WC  1/2 WC  1/2 WC  1/2 WC  1/1 WC  1/1 WC  1/1 WC |   This medium was invented by ourselves based on the composition of GG medium (von Stosch & Fecher 1979. J Phycol 15:233-243), but making use of the stock solutions of WC medium. These can simply be added in the proportions specified above to obtain PM. The pH of PM culture medium should be between 4.0 and 4.5 after autoclaving. Lower the pH with 1 N HCl. Otherwise, the medium can be prepared in the same way as specified above for WC. |