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We use this protocol and it's working

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WC Medium

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DISCLAIMER

This is a protocol used in the Becks Lab, 2023. The modification used here is the amount of Phosphorous added.

ABSTRACT

WC Medium modified from Guillard and Lorenzen (1972)

Stock Solution preparation

1 The stock solutions don't need to be autoclaved after preparation. Make sure to use volumetric flasks to ensure the right concentrations of the salts and transfer the solution to autoclavable flasks and store it in the fridge. Remember to start the preparation with less than 1L of MilliQ water, and after adding the salts complete the volume to 1L.

1.1 Stock solution **A**: **36,80g of ($CaCl_2$+$2H_2O$)** in 1L of MilliQ water

1.2 Stock solution **B**: **37g of ($MgSO_4$+$7H_2O$)** in 1L of MilliQ water

1.3 Stock solution **C**: **12,60g of ($NaHCO_3$)** in 1L of MilliQ water

1.4 Stock solution **D**: **11,40g of (K_2HPO_4 + H_2O)** in 1L of MilliQ water

1.5 Stock solution **E**: **85 g of ($NaNO_3$)** in 1L of MilliQ water

1.6 Stock solution **F**: **21,20 g of ($Na2SiO_3$ + $5H_2O$)** in 1L of MilliQ water

1.7 Stock solution **G (Supplement elements)**: add the following salts in 1L of MilliQ water:
- 4,36 g of (Na_2EDTA)
- 3,15 g of ($FeCl_3$ + $6H_2O$)
- 0,010 g of ($CuSO_4$ + $5H_2O$)
- 0,022 g of ($ZnSO_4$ + $7H_2O$)
- 0,010 g of ($CoCl_2$ + $6H_2O$)
- 0,180 g of ($MnCl_2$ + $4H_2O$)
- 0,006 g of (Na_2MoO_4 + $2H_2O$)
- 1,0 g of (H_3BO_3)

1.8 Stock solution **H (Vitamin Mix)**: add the following in 1L of MilliQ water:
- **Thiamine hydrochloride (Vitamin B1)**: 0,1 g of ($C_{12}H_{17}ClN_4OS$)
- **Biotin**: 0,0005 g of ($C_{10}H_{16}N_2O_3S$)

- **Cyanocobalamin (Vitamin B12)**: 0,0005 g of ($C_{63}H_{88}CoN_{14}O_{14}P$)

Aliquot the solution in 15 mL falcon tubes, and freeze them until used at -20 ºC.

Medium preparation (for 1L of WC)

2 Weigh **0,115 g** of **TES Buffer** ($C_6H_{15}NO_6S$) and add it to a volumetric flask with almost 1L of MilliQ water

3 Add **1 mL** of each of the stock solutions **A** to **G** to the medium preparation bottle.

4 **Complete** the volume until 1 liter with MilliQ water

5 Transfer the prepared medium to an autoclavable flask, labeled with autoclaving tape.
On the label put the information about the liquid contained (WC medium), the date, the person responsible for the preparation, and a box to check when adding the solution **H** (Vitamin Mix).

6 **Autoclave** the medium at 121ºC and let it cool down to room temperature.

7 In a clean bench: syringe filter 1 mL of the defrosted **solution H (Vitamin Mix)**, add it to the medium (1 ml per Liter), and check the box on the tape.