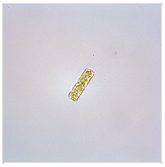

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Preparing phytoplankton samples for elemental carbon and nitrogen analysis

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

Protocol for the preparation of phytoplankton samples for elemental carbon and nitrogen analyses.

ATTACHMENTS

POC PON protocol.docx

PROTOCOL CITATION

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KEYWORDS

POC PON, elemental analysis, diatoms

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MATERIALS

NAME	CATALOG #	VENDOR
Distilled Water		
Tissue Culture Flask, 50ml, 10/Cs	TCF70-50.SIZE.1PK	Bio Basic Inc.
Hydrochloric acid	H1758	Sigma Aldrich
6-well plate		Corning
Vacuum Manifold Filtration Unit	MSVMHTS00 or equivalent	Millipore
Whatman GFF filters 25mm	Z242489	Sigma Aldrich

Preparing GFF filters 2d

1 Wrap up to 20 Whatman GFF filters in aluminium foil and precombust them for 6 hours at 450degrees C, store filters airtight afterwards

2 Weigh precombusted filters and store labelled and individually (e.g. in 6-well microtitre plates)

Preparing phytoplankton cultures 5d

3 Grow 45 mL phytoplankton cultures in 50 mL flasks until they reach mid-exponential phase, culture are dense enough when some colour is visible

Applying samples onto filters 1d

4 1 mL used of each sample used for cell counting (e.g. with fluorescence plate reader/FlowCam/FlowCytometry? Microscopy)

5 Use a vacuum filtration unit to apply the phytoplankton samples onto the GFF filters
From the remaining 44 mL per sample, 20 mL each used for 2 replicate filters
Apply the ample onto filter using a pipette, carefully check volume
Wash each the edge of the filter unit and the filter with 10mL dest. water

6 Filters with biomass can be stored again in the 6-well plates,
Let filters dry with loose lid overnight

Preparation of filters for elemental analysis 5d

7 Dry filters at 40 degrees C for 2-3 days
Weigh all dried filters again and calculate the net weight of the dry biomass for each filter

8 For work with an elemental analyzer that measures total carbon content, the inorganic carbon content has to be removed: (this can take place in the 6-well plates
Soak filters with 200uL dest. water, then apply 100uL 2M HCl onto each filter to remove any inorganic carbon from the filters
Let filters dry overnight

9 Filters are now ready for elemental analysis