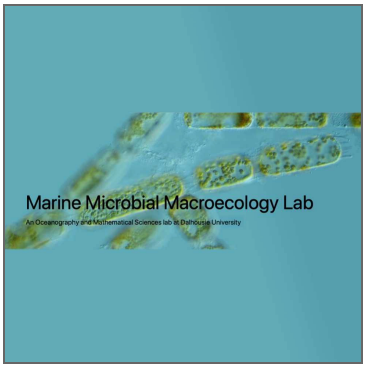

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Protocol status: Working
We use this protocol and it's working

Created: Jan 08, 2023

Basic pipet technique training

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ABSTRACT

Here we adopt the molybdate colorimetric reaction from our Total particulate phosphorus (TPP) measurement as a training material for our new lab members to learn basic pipetting technique.

CITATION

YY Hu, AJ Irwin, ZV Finkel (2022). Improving quantification of particulate phosphorus. Limnology and Oceanography: Methods.
LINK
10.1002/lom3.10517

CITATIONS

YY Hu, AJ Irwin, ZV Finkel. Improving quantification of particulate phosphorus
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PROTOCOL MATERIALS

Ammonium molybdate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**09878-100G

Step 6

Ascorbic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5960-100G

Step 7

SAFETY WARNINGS

Acidic reagents need to be neutralized before dumped into the sink.

Preparing standard working solutions

2h

1 Date:

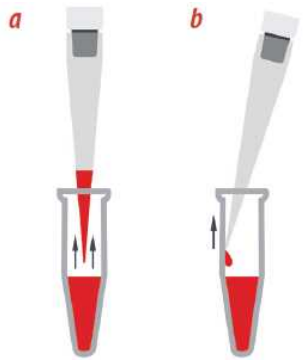
Trainee:

2 Need the following materials:

	Supply	Check
	Microtube rack	
	17 X 2 mL microtubes	
	MilliQ water container	
	10 uL pipet	
	100 uL pipet	
	1000 uL pipet	
	10 uL pipet tip	
	100 uL pipet tip	
	1000 uL pipet tip	
	Vortex mixer	
	Sharpies	
	Primary stock solution of KH2PO4	
	MilliQ	

3 How to aspire and dispense with pipet

(a) When aspiring solution, ensure the pipette to be held vertically.
(b) When dispensing, ensure you hold the pipette at an angle (10-45°). Working to these angles ensures the desired liquid amount is drawn into the tip properly and that all of the liquid is fully dispensed without leaving any residue in the tip.



https://www.americanlaboratory.com/914-Application-Notes/240482-Ten-Tips-for-Proper-Pipetting

Note

Always pay attention to the pipet tip, check if there is air bubble in the tip or slightly leaking on the tip.

4 Standard working solutions

	Concentration of KH2PO4 primary stock	Unit

	KH2PO4	Primary (uL)	Check	MilliQ (uL)	Check
	S1	0		500	
	S2	2		498	
	S3	5		495	
	S4	10		490	
	S5	25		475	
	S6	50		450	
	S7	75		425	
	S8	100		400	

Prepare working reagents

2h

5 Need the following materials:

	Supply	Check
	50 mL Falcon tube (3)	
	Transfer pipet (1)	
	Scoopula (1)	
	Anti-static weighting plate (2)	
	MilliQ	
	Kimwipe	
	Chamber balance	
	Graduated cylinder (1X25 mL)	
	Goggles	

6 [M] 2.5 % ammonium molybdate reagent

Ammonium molybdate **Sigma Aldrich** **Catalog #**09878-100G

6.1 In an anti-static weight plate, weigh ~0.25 g ammonium molybdate, transfer into the labelled falcon tube.

	Actual weight (g)

6.2 Measure 10 mL MilliQ (use transfer pipet for the final topping)

6.3 Rinse the residue on the weighing plate into the falcon tube with the MilliQ.

6.4 Transfer all remaining MilliQ into the falcon tube

7 [M] 10 % ascorbic acid reagent (avoid light exposure)

Ascorbic acid **Sigma Aldrich Catalog #**A5960-100G

7.1 In an anti-static weight plate, weigh ~1 g ascorbic acid, transfer into the labelled falcon tube.

	Actual weight (g)

7.2 Measure 9 mL MilliQ (use transfer pipet for the final topping)

7.3 Rinse the residue on the weighing plate into the falcon tube with the MilliQ.

7.4 Transfer all remaining MilliQ into the falcon tube

8 Sulfuric acid reagent:

8.1 Measure 17 mL MilliQ (use transfer pipet for the final topping) and transfer into a Falcon tube

8.2 Carefully add 1 mL [M] 18 M concentrated sulfuric acid into the water

9 Vortex ammonium molybdate and ascorbic acid solutions.

10 Use graduated cylinder, measure and transfer 6 mL [M] 2.5 % ammonium molybdate reagent into the sulfuric acid tube.

11 Use graduated cylinder, measure and transfer 6 mL [M] 10 % ascorbic acid reagent into the sulfuric acid tube.

12 Vortex the reagent.

Colorimetric measurement

2h

13 Preheat incubator/shaker to 37 °C

Equipment	
SHAKING INCUBATOR	NAME
71L	TYPE
Corning® LSE™	BRAND
6753	SKU

14 Add 500 µL reagent to each standard working solution.

15 Vortex each tube.

16 Incubate at 37 °C for 01:00:00 while shaking at 200 rpm 1h

17 Load microplate with 250 uL reactant from each tube, duplicate.

18 Layout in the microplate:

	1	2	3	4
A	S1	S1	S1	S1
B	S2	S2	S2	S2
C	S3	S3	S3	S3
D	S4	S4	S4	S4
E	S5	S5	S5	S5
F	S6	S6	S6	S6
G	S7	S7	S7	S7
H	S8	S8	S8	S8

19 Turn on microplate reader

Equipment

Varioskan LUX Multimode Microplate Reader NAME

Thermo Fisher BRAND

VL0L00D0 SKU

20 Setup layout and method

	A	B
	Shake duration	00:00:05
	Shaking type	Continuous
	Shaking force	High
	Shaking speed [rpm]	600
	Wavelength [nm]	820
	Measurement Time [ms]	100

21 Place microplate onto the plate holder

22 Read the plate

23 Export data to Excel sheet

Calculation

24 In the Excel sheet, subtract the average absorbance at 820 nm of the blank standard replicates from the absorbance at 820 nm of all other standard working solutions.

This gives the corrected absorbance from each well.

25 Calculate the concentration of each standard working solution
Unit for each content:
Primary concentration g/L
Primary volume uL
MilliQ volume uL
Standard working solution g/L
Phosphate in standard working solution uM

26 Y-axis: Corrected absorbance
X-axis: Phosphate (uM)
Calculate slope (S), intercept (b), coefficient of determination (R^2), standard deviation (S_y) of the absorbance from the four replicates of the blank, and LOD = 3.3 X S_y/S

	Parameter	Value
	S	
	b	
	R2	
	Sy	

	Parameter	Value
	LOD	

Review

27 What information does LOD provide?

28 Is it necessary to be aware of the order when mixing water with concentrated sulfuric acid?

Clean-up

29 Neutralize acidic chemicals with baking soda in a plastic beaker

30 Rinse microtubes and microplate with tap water

31 Dispose microtubes and microplate into recyclable garbage bin

32 Dispose pipet tips into sharp garbage container

33 Rinse graduated cylinder, Falcon tubes and MilliQ container with DI water, place them into a basket to air-dry