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Version 1

Basic pipet technique training V.1

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Protocol status: Working

We use this protocol and it's working

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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.

[10.1002/lom3.10517](https://doi.org/10.1002/lom3.10517)

[LINK](#)

Protocol materials

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

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

Safety warnings

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



Preparing standard working solutions

2h

1 Date:

Trainee:

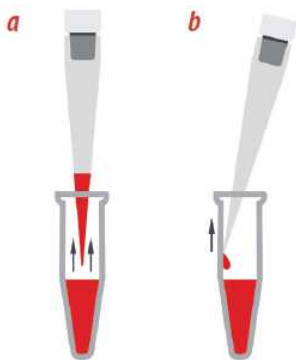
2 Need the following materials:

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 KH_2PO_4	
MilliQ	

3 How to aspire and dispense with pipet

(a) When aspirating 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 KH ₂ PO ₄ primary stock	Unit

	KH ₂ PO ₄	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 **Merck MilliporeSigma (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 **Merck MilliporeSigma (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 Mass Percent 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



A	B
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 $LOD = 3.3 \times S_y/S$

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

Citations

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