

Sediment Phosphorus Extraction

Run Week Task Guide



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INTRODUCTION

This run week task guide discusses only the essential information for performing a sediment phosphorus extraction run (PER); mainly, the pre-run setup and run task work. Keep a copy of this guide handy during a PER to quickly reference the next tasks and steps.

The topics in this guide are taken from the *Performing a Phosphorus Extraction Run: User's Guide*. Be sure to read and understand the user's guide before performing a PER, or using this task guide.

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SAFETY PROCEDURES

You must follow standard laboratory safety procedures when performing a PER.

Most reagents and equipment used in this process are comparatively safe. The diluted reagents are relatively weak (though the 1 N HCl may sting skin on contact), and the equipment, when properly used, does not easily catch loose clothing or jewelry.

Eyes

Wear safety goggles when around chemicals, even if you are not directly handling them. For example, when one person is at the fume hood creating solutions, and another person is at the lab bench crushing sediment, both workers must be wearing safety goggles.

Danger! Potential eye injury or blindness. **Do not wear contacts while in the lab.** Certain chemicals' fumes or accidental splash-back may cause the contacts' chemical composition to change (melt or harden), leading to possible eye damage, and even blindness.

Face and Body

- When working at the fume hood, position the sliding glass panels to protect your face and body.
- Wear gloves and long-sleeved shirts when handling chemicals.
- Thoroughly wash hands immediately after handling chemicals.
- Wear sturdy, closed-toed shoes.
- Remember that certain chemicals may permanently stain clothing. Always wear lab coats to protect your clothes, and do not wear expensive or dressy clothes when working in the lab.
- To prevent injuries due to accidents, always use a lab cart to transport chemicals and breakable objects (such as glass bottles or lab equipment) between all rooms and within a room.

Caution! Possibility of injury. Do not wear any clothing, jewelry, or hairstyles that could get entangled in moving machinery. Secure long hair; do not wear extremely loose clothing, dangling earrings, necklaces, and bracelets; remove rings from fingers.

PRE-RUN TASKS

As you normally start a run on a Monday, the following four tasks must be completed by the previous Friday.

Weigh Samples

Weigh the test and control samples into the run's centrifuge tubes:

1. Before weighing samples, ensure that the digital scale is thoroughly cleaned and accurately calibrated.
2. Measure out each sample on a piece of creased weighing paper so it is easier to pour the sample into the centrifuge tube.
3. Ensure that each sample's weight is close to 0.100 g.
4. Record each sample's weight on the run's Excel sheet.

Calibrate Repipets

Calibrate three repipets:

1. Calibrate two repipets at 10 mL for the Monday dispense of C/B and MgCl_2 solutions.
2. Calibrate one repipet at 13 mL for the Tuesday dispense of 1 N HCl.

Check Reagent Readiness

Prepare all solutions in at least the necessary minimum quantities, and verify their quality:

- Set up and calibrate the pH meter (pH buffer standards are stored in the stockroom).
- Check the pH of each solution. Use concentrated HCl and concentrated NH_4OH for adjusting.
- Use the *Phosphate Contamination: HACH Kit Testing* (see page 5) to check for phosphate contamination.

Perform General Run Preparation Tasks

Verify that these tasks are completed before starting a run:

- Ensure that the crucibles, reagents, and centrifuge tubes and trays are labeled with the run's code.
- Ensure that all equipment and instruments are clean, acid-washed if necessary, and ready for use.
- Ensure that full Nalgene's of water and 1N HCl are near the sink.
- Ensure that all lab partners have a lab book for writing notes.
- Confirm the work schedule with all lab partners.

Note: As Monday is generally a 12–14 hour workday, plan to start work between 7–8 a.m. The subsequent run workdays are shorter, and work can begin later (though no later than 9 a.m.).

MINIMUM RUN REAGENT AND CHEMICAL QUANTITIES

The Minimum Run Quantities table lists the seven reagents and chemicals used during a single processing run, their necessary minimum quantities, and required pH level. Be sure to verify all run reagents and chemicals are correctly prepared and phosphate-free before using them in an extraction run.

Table 1: Minimum Run Quantities

Name	Formula	Minimum Quantity	pH
Citrate bicarbonate	C/B	700 mL	7.6
De-ionized water	DI	960 mL	NA
Hydrochloric acid	1 N HCl	1300 mL	NA
Magnesium chloride	MgCl ₂	1440 mL	8.0
Magnesium nitrate	Mg(NO ₃) ₂	48 mL	NA
Sodium acetate	Na acetate	48 mL	4.0
Sodium diethionite	Na ₂ S ₂ O ₄	4 g	NA

SIGNS OF REAGENT PHOSPHATE CONTAMINATION

The reagents must be phosphate-free to ensure valid test results. Hold samples in clear glass containers against white paper, in a well-lit environment, to determine the reagent's color.

Signs of phosphate contamination are:

- The magnesium chloride solution has a yellow tinge after adding the sulfuric acid and ammonium molybdate solutions.
- The ammonium molybdate solution used to make the mixed reagent has a yellow tinge, instead of being clear. Clear ammonium molybdate indicates a phosphate-free solution.
- The mixed reagent has a blue tinge; this occurs when the ammonium molybdate solution is contaminated with phosphate.
- A reagent sample develops a blue tinge when tested with the HACH kit.

Phosphate Contamination: HACH Kit Testing

To perform the HACH kit test:

1. Follow the low-range test instructions on pages 1–2 of the HACH kit's instruction manual.
2. Hold the control and sample test tubes against a white background (such as a piece of paper) to discern slight color differences.
3. Determine if the sample reagent is clear or has a blue tinge.
 - **Sample is clear:** Indicates there is no phosphate contamination, and the reagent can be used.
 - **Sample has a blue tinge:** Indicates phosphate contamination; discard the reagent and acid-wash all bottles and equipment that have come in contact with the solution.

PERFORMING A PER: OVERVIEW

The PER process extracts specific types of phosphorus (oxide-bound, authigenic, detrital, and organic) from ocean floor sediment samples for subsequent analysis with the Lachat ChemQuick® 8500 Series 2 FIA analyzer. Each phosphorus type is suspended in solution in a numbered bottle, and the numbered bottles correspond to a step in the Lachat analysis. There are five Lachat analysis steps (four phosphorus analysis steps and one standards analysis step), but only three trays of sample bottles, as the fourth step is retained in the centrifuge tubes.

The Schedule Details table shows the phosphorus extraction schedule by type, workday, container, and corresponding Lachat analysis step.

Table 2: Schedule Details

Phosphorus Type	Extraction Workday(s)	Contained In	Lachat Analysis Step
oxide-bound	1–2	30 mL Step 1 bottles	Step 1
authigenic	2	60 mL Step 2 bottles	Step 2
detrital	2–3	20 mL Step 3 bottles	Step 3
organic	3–5	centrifuge tubes	Step 4
phosphorus standards	NA	standards 40 mL bottles	Step SA

The Procedure Term Definitions table defines how-to techniques for performing specific PER steps of the extraction procedures.

Table 3: Procedure Term Definitions

Procedure Term	Definition
Prepare samples for shaking	Lay the four shaker trays with samples horizontally onto the shaker, in two stacks of two trays each. Secure the trays onto the shaker with tape or small bungee cords.
Cap and tap	With 1–3 tubes in each hand, briskly pound the tubes' cap end on the counter to mix the sediment and solution. Place tubes in tray. Immediately after completing a tray, place each tray horizontally on the shaker to prepare the samples for shaking; this helps keep the sediment suspended in solution.
Centrifuge the tubes	Centrifuge the tubes on the 3 setting for 7 minutes. If all 48 tubes cannot be centrifuged simultaneously, multitask by centrifuging one batch while decanting and refilling another batch.

MONDAY

Task: Begin extracting oxide-bound phosphorus

This procedure begins extracting most of the oxide-bound phosphorus from the sediment into solutions contained in the Step 1 bottles. This is generally a 12–14 hour day, depending on the number of workers, number of repipets, and centrifuge capacity (48 tubes or less). Plan to start work between 7–8 a.m.

Supplies needed:

- 30 mL Step 1 bottles and tray
- Centrifuge
- Centrifuge tubes and tube trays
- C/B solution and 1 L bottle
- DI water in a 1 L bottle
- Graduated cylinder
- Repipet calibrated to dispense 10 mL
- Repipet filled with MgCl_2 , calibrated to dispense 10 mL
- Shaker table and securing equipment
- $\text{Na}_2\text{S}_2\text{O}_4$

Pre-Run Tasks

Perform these tasks within 15 minutes of starting a run:

- Filter the C/B reagent if it is cloudy, using the sediment sifter and a micron mesh swatch.
- Weigh out 4 grams of $\text{Na}_2\text{S}_2\text{O}_4$; store in a labeled, tinted glass vial.

1. Set up centrifuge tubes in shaker trays, remove caps, and check that there is sample in each tube.
2. Measure 700 mL C/B solution into the C/B 1 L bottle using the graduated cylinder.
3. Add in 4 g $\text{Na}_2\text{S}_2\text{O}_4$ to the C/B solution; cap and shake.
4. Perform steps 4 through 7 immediately after mixing this solution, as quickly as possible.
5. Attach the repipet. Prime the repipet to remove any air bubbles, using as little solution as possible.

Note: Retain at least 50 mL of the solution for the standards.

6. Dispense 10 mL C/B into each sample tube, and cap the tubes.
7. Prepare samples for shaking (see page 9).
8. Set the shaker on a timed shake of 300 minutes (6 hours) and 90 rpm.

Note: While the samples shake, clean and prime the C/B repipet for the step 15 DI dispensement.

9. Centrifuge the tubes (see page 9).
10. Pour off the C/B solution from each tube into its corresponding Step 1 plastic bottles.
11. Prime the MgCl_2 repipet, and add 10 mL MgCl_2 solution to each sample.
12. Cap and tap (see page 7).
13. Shake at 90 rpm for 120 minutes (2 hours).
14. Centrifuge the tubes (see page 7).
15. Pour off the MgCl_2 from each tube into its corresponding Step 1 plastic bottle.

16. Dispense 10 mL DI water into each sample.
17. Cap and tap.
18. Shake at 90 rpm for 120 minutes (2 hours).

This is the last task of the day; you can leave after confirming the shaker table is correctly programmed and operating.

Important! Be sure to alert the night lab monitor that the shaker table is on and complete the required form. Otherwise, the night lab monitor will power off the table.

TUESDAY

You perform three extraction tasks today for oxide-bound, authigenic, and detrital phosphorus.

Task 1: Finalize extracting oxide-bound phosphorus

This procedure completes the oxide-bound phosphorus extraction process started on Monday.

Supplies needed:

- 30 mL Step 1 bottles and tray
- Centrifuge
- Centrifuge tubes and tube trays
- Shaker table and securing equipment

1. Shake the samples for 15 minutes to loosen up the settled residue.
2. Centrifuge the tubes.
3. Pour off the solution from each tube into its corresponding Step 1 plastic bottle.

All of the oxide-bound phosphorus is extracted. Go to task 2 on the next page.

Task 2: Extract authigenic phosphorus

This procedure extracts all the authigenic phosphorus from the sediment into solutions contained in the Step 2 plastic bottles.

Supplies needed:

- 60 mL Step 2 bottles and tray
- Centrifuge
- Centrifuge tubes and tube trays
- Repipet filled with MgCl_2 , calibrated to dispense 10 mL
- Repipet filled with Na acetate, calibrated to dispense 10 mL
- Shaker table and securing equipment

1. Dispense 10 mL Na acetate into each sample.
2. Cap and tap.
3. Remove the cap to release the CO_2 , then recap.
4. Repeat steps 2 and 3.
5. Prepare samples for shaking (see page 7).
6. Shake on 90 rpm for 300 minutes (5 hours).
7. Centrifuge the tubes.
8. Pour off the Na acetate solution from each tube into its corresponding Step 2 plastic bottle.
9. Dispense 10 mL MgCl_2 into each sample.
10. Cap and tap.
11. Prepare samples for shaking.
12. Shake on 90 rpm for 120 minutes (2 hours).
13. Centrifuge the tubes.
14. Pour off the MgCl_2 solution from each tube into its corresponding Step 2 plastic bottle.
15. Repeat steps 9 through 14, with a change at step 12. For step 12, shake on 90 rpm for 60 minutes (1 hour).
16. Repeat step 15.

All the authigenic phosphorus is extracted. Note that each bottle contains 40 mL total of solution: 10 mL Na acetate and 30 mL MgCl_2 . Go to task 3 on the next page.

Task 3: Begin extracting detrital phosphorus

This procedure begins the extraction of detrital phosphorus from the sediment.

Supplies needed:

- Centrifuge tubes and tube trays
- Repipet filled with 1 N HCl, calibrated to dispense 13 mL
- Shaker table and securing equipment

1. Dispense 13 mL HCl into each sample.
2. Cap and tap.
3. Shake at 90 rpm for 960–1200 minutes (16–20 hours).

Important! Be sure to alert the night lab monitor that the shaker table is on and complete the required form. Otherwise, the night lab monitor will power off the table.

This is the last task of the day. You can leave after confirming the shaker table is correctly programmed and operating and all necessary night lab procedures have been completed.

WEDNESDAY

You perform two extracting tasks today for detrial and organic phosphorus.

Task 1: Finalize extracting detrial phosphorus

This procedure completes the extraction of the detrial phosphorus from the sediment into solutions contained in the Step 3 bottles.

Supplies needed:

- 20 mL Step 3 bottles and tray
- Centrifuge
- Centrifuge tubes and trays

1. Centrifuge the tubes.
2. Pour off the HC1 from each tube into its corresponding Step 3 bottle.

All the detrial phosphorus from the sediment is extracted. Go to task 2 on the next page.

Task 2: Bake samples in the lab oven

This procedure begins the extraction of the organic phosphorus from the sediment. This task is the first of four procedures for extracting organic phosphorus.

Supplies needed:

- 1 mL pipette
- 50% by volume $\text{Mg}(\text{NO}_3)_2$ solution
- Aluminum pie plates
- Crucibles and carrying trays
- DI water
- Laboratory oven
- Squirt bottle

1. Use a squirt bottle filled with water to flush out sample residue from each tube into its corresponding crucible.

Note: Try to use as little water as possible (1–2 mLs) to reduce the drying time.

Important! Do not discard the sample's centrifuge tubes.

2. Place the crucibles on the aluminum pie plates.
3. Place the pie plates in the lab oven on different racks, ensuring there is adequate heat flow around each plate.
4. Dry the samples in the lab oven at 80–90°C for 2–6 hours.

Note: Drying time varies depending on the amount of water in the crucible. Check the samples every 30–60 minutes to determine if they are done.

Important! Ensure that the oven does not exceed 95°C, as this causes the water to boil.

5. Remove the samples from the oven and allow them to cool for ~30 minutes.
6. Pipette 1 mL of 50% by volume $\text{Mg}(\text{NO}_3)_2$ into each crucible.
7. Dry samples again in the oven at 60°C.
8. Finish the evaporation process.
 - a. Remove the samples from the oven and allow them to cool for ~15–30 minutes.
 - b. Place the crucibles on the carrying trays and cover with aluminum foil, to prevent product loss and contamination.
9. Ensure the lab oven is turned off.

Important! If you perform step 7 near the end of the workday, you must dry the samples overnight. Program the oven to automatically shut off 4–6 hours from the drying start time. Be sure to alert the night lab monitor that the lab oven is on and complete the required form. Otherwise, the night lab monitor will power off the lab oven.

THURSDAY

You perform two tasks today to continuing extracting organic phosphorus from the sediment.

Task 1: Bake samples in the muffle furnace

This procedure ashes the samples.

Supplies needed:

- Aluminum foil
- Crucibles and carrying trays
- Muffle furnace
- Sharpie pen

1. Place the crucibles in the muffle furnace.
2. Turn on the furnace and set the temperature to 550°C.

Note: It generally takes 30 minutes to reach this temperature; the furnace emits three short beeps when the programmed temperature setting is met.

3. Bake the crucibles for 2 hours at 550°C.
4. Cool the crucibles for 60–90 minutes.
 - a. Turn off the oven, and keep the door closed for 30 minutes for the initial cool-down.
 - b. Open the door and allow the crucibles to cool further (another 30–60 minutes) until they can be safely handled.
5. Renumber each crucible with a Sharpie pen as you remove it from the oven.

Note: Be careful not to mishandle the crucible and spill the ashed product.

6. Place the crucibles back on the carrying trays, and cover with aluminum foil to prevent product loss and contamination.

The ashed samples are prepared for the third organic phosphorus extraction task. Go to task 2 on the next page.

Task 2: Shake samples in centrifuge tubes

This procedure puts the ashed samples into a solution.

The process to prepare the centrifuge tubes generally takes one person 2–3 hours to complete.

Supplies needed:

- 50 mL glass beaker
- 1000 µl Eppendorf pipet and pipet tips

Tip: Cut pipet tips off at an angle; this makes the process easier and faster.

- Centrifuge tubes, with caps off
 - Crucibles and carrying trays
 - Repipet filled with 1 N HCl, calibrated to dispense 13 mL
1. Using the 1000 µl Eppendorf pipet, dispense 1 mL HCl into each crucible, and let this sit to soften the ashed sediment.
 2. Using the repipet, dispense 13 mL HCl into the 50 mL glass beaker.
 3. Transfer most of the ashed sediment from the crucible into the appropriate centrifuge tube.
 - a. Using the Eppendorf pipet, squirt another 1 mL HCl into a crucible.
 - b. Use the pipet tip to gently mix the acid with the softened ashed sediment, forming a solution. For resistant residue, use the pipet like a pressure nozzle to resuck and resquirt the solution a few times, and break down the resistant residue.
 - c. Pipet the acid and ashed sediment solution into the appropriate centrifuge tube.
 - d. Repeat sub-steps a through c until only a small amount of sediment remains in the crucible bottom.
 4. Transfer the remaining sediment into the centrifuge tube.
 - a. Hold crucible over centrifuge tube in one hand, with thumb and index finger holding crucible over tube held by middle, ring, and pinkie finger.
 - b. With the other hand, squirt HCl from pipet into crucible and tilt wrist forward so that residue swishes into tube.
 - c. Repeat sub-steps a and b until the crucible is clean.
 5. Clean out the pipet tip with HCl, pipetting it into centrifuge tube.
 6. Pour the remaining HCl from the 50 mL beaker into centrifuge tube; this means each tube will have ~14 mLs HCl. Cap the tube.
 7. Repeat steps 2 through 6 for each sample.
 8. Prepare samples for shaking.
 9. Shake at 90 rpm for 960 minutes (16 hours).

Important! Be sure to alert the night lab monitor that the shaker table is on and complete the required form. Otherwise, the night lab monitor will power off the shaker table.

This is the last task of the day. You can leave after confirming the shaker table is correctly programmed and operating and all necessary night lab procedures have been completed.

FRIDAY

Task: Finalize extracting organic phosphorus

This procedure completes the extraction of the organic phosphorus from the sediment.

Supplies needed:

- Centrifuge
- Centrifuge tubes and trays
- Sharpie pen and white tape

1. Centrifuge the tubes.
2. Create labels indicating *Ready for Step 4* and attach to trays.
3. Place the tubes in the trays.

All the organic phosphorus is extracted. This is the final PER step; you can now perform the Lachat analysis on the extracted phosphorus solutions.

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