
Total Phosphorus
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SUMMARY

This procedure is used for measuring total phosphorus in water samples using Hach TNT 843 low range phosphorus kits.

EQUIPMENT AND SUPPLIES

Hach DR2800 Spectrophotometer	1000µl pipette
2000µl pipette	Hach TNTplus 843 kits
200µl pipette	Hach DRB200 heating blocks
kimwipes	alcohol swabs
sample racks	timers (at least 2)
pipette tips	100mL Volumetric Flasks
Eye Droppers	Ultrapure water (Type 1)
Acid washed squeeze bottle	Gloves
Analytical balance with at least 2 decimal places	

NOTES

- All glassware (with the exception of the Hach kit vials) used for samples, standards, or blanks needs to be acid-washed prior to use.
- Samples need to be analyzed as soon as possible (within 6 hours of receipt at the lab).
- Never pour leftover chemicals down the drain or in the trash. There are appropriate receptacles located in the hood in Room 105.
- Check the date balances were last calibrated before using. They should be calibrated by a professional on an annual basis and checked using calibrated weights monthly.

- Pipettes used in this procedure need to be calibrated annually and checked using a balance on a monthly basis or as problems arise.
- Wear gloves at all times during procedure!

REAGENTS

1. All chemicals used in this procedure are included in the Hach TNT 843 kit. Use care when pipetting these reagents and dispose of appropriately.

STANDARDS

1. 50mg/L PO₄⁻³ (NIST) stock solution for making standards is purchased from the Hach company (product # 17149). This solution is stable in the refrigerator for about six months after opening or until expiration date on bottle (whichever comes first).
2. For all standards, mix stock in ultrapure (type 1) water. These should be prepared no more than 24 hours before each sampling event. Place a 100mL volumetric flask on the analytical balance, and tare. Add amount of 50mg/L stock standard given below using a new eye dropper. Use the squeeze bottle to dilute to mark with type 1 ultrapure water. Store in acid washed, glass, amber bottle. 0ppm is simply ultrapure water.

0.5ppm: 1g stock solution

1.000ppm: 2g stock solution

2.500ppm: 5g stock solution

4.00ppm: 8g stock solution .

These standards are run by lab personnel at the beginning of each sampling day to test the efficiency of laboratory equipment. If the standards are off by more than 10%, recalibrate spec and rerun standards. If they are still off, record the values and make sure that linearity is retained in the event that sample values need adjusted at a later date (ex. purchased standards are also off by same amount).

3. In addition to prepared standards, a wastewater standard (Hach # 2833249) and drinking water standard (Hach # 2833049) are run through as samples to

test the accuracy of the volunteer running the samples. These standards need to be within 10% of their expected values in order for accuracy to be considered achieved.

SAMPLE PREPARATION AND STORAGE

Sample are collected in coolers containing ice packs to keep them as cool as possible in the field and should be stored at 4° C immediately upon arrival in the lab. Samples are to be analyzed within 6 hours of arrival at the lab. If it is not possible to analyze samples within this time range, samples must be frozen. Freezing samples should only happen in cases of extreme emergency or complete equipment failure.

SAMPLE ANALYSIS

Digestion:

1. Make sure vial is marked with correct sample number.
2. Check to make sure heating blocks are on and heated to 100° C and spectrophotometer is on and reads “please insert barcode cuvette”. If this is not the case, find a lab manager and inform them of the issue. If you are unfamiliar with using a pipette, please see a lab manager. Please note: Check the lot numbers on the sample box prior to starting a new box. Whenever the lot number on the sample vials changes, please rerun a blank and a standard before running any more samples. If the values are off or you are unsure how to do this, please see a lab manager.
3. Unscrew cap from the vial, and gently place on counter thread side up. Make sure that you keep the reagent caps in order. In case of a lot number change within a batch, it is best that a cap remain with its original vial.
4. Pipette 2000µL (2mL) of sample into the reagent vial using a new pipette tip and the 2000µL (green top) pipette. Place in test tube rack.
5. Repeat steps 3 and 4 for the next 14 samples. Once you have 15 vials, carefully remove the protective foil from the vial lid. Flip the cap over the

vial so that the side containing the reagent is facing the vial opening.

Screw cap tightly onto the vial.

6. Invert the vial 2 to 3 times to remove the reagent from the cap. Verify that there is no reagent left in the cap.
7. Insert vials into the heating block, making sure to maintain the sample order. Close the lid. Try to minimize the amount of time the heater blocks are left open to avoid unnecessary fluctuations in temperature.
8. Press start to begin the 60 minute digestion.
9. Repeat steps 2 through 7 for the next 15 samples. We have enough heating blocks to have 4 batches going at a time.
10. After the timer goes off, carefully remove the vials from the heater block. Place the vials in a rack, and place the rack in the refrigerator for 15 minutes or until they are around room temperature. Press start again on the heater block to keep it heated and reset the timer.

Color Reaction:

1. Pipette 200 μ L of Reagent B into the cooled sample vial using the 200 μ L (yellow top) pipette. Try to minimize the amount of time the lid is off of the reagent container. It is light sensitive.
2. Screw the gray DosiCap C onto the vial.
3. Invert the vial 2 to 3 times to remove the reagent from the cap. Verify that there is no reagent left in the cap.
4. Set timer for 10 minutes.
5. While reaction is occurring, wipe down vials with an alcohol swab to remove and dust, fingerprints, etc.
6. When timer beeps, again invert the vial 2 to 3 times to mix.
7. Sample one is used to blank the machine. Please see a lab manager when this sample is ready. Remove the cover from spec. Place the vial into the cell holder, making sure to align barcode with arrow, and quickly replace cover. This test is time sensitive! Read results as quickly after reaction time as possible.

8. Record value on the data sheet. Note: If the machine reads **Under Range**, make sure to record that on the data sheet. If the machine reads **Over Range**, put sample aside to be diluted and rerun. For dilution: using 1000 μ L pipette (blue top), pipette 1000 μ L of sample and 1000 μ L of ultrapure water into a new vial and repeat steps all prior steps. Make sure to mark the sample on the sheet as diluted. Retain both undiluted and diluted values.

CLEAN UP

Turn off heater blocks. Throw used pipette tips, kimwipes, and alcohol swabs in the trash. Place capped reaction vials on designated disposal bin. Straighten bench space.