

# Phosphorus, Reactive (Orthophosphate)

DOC316.53.01119

USEPA<sup>1</sup> PhosVer 3 (Ascorbic Acid) Method<sup>2</sup>

Method 8048

0.02 to 2.50 mg/L PO<sub>4</sub><sup>3-</sup>Powder Pillows or AccuVac<sup>®</sup> Ampuls**Scope and Application:** For water, wastewater and seawater<sup>1</sup> USEPA Accepted for reporting for wastewater analyses. Procedure is equivalent to USEPA and Standard Method 4500-P-E for wastewater.<sup>2</sup> Adapted from *Standard Methods for the Examination of Water and Wastewater*.

## Test preparation

### How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

**Table 1 Instrument-specific information**

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

#### Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

#### Collect the following items:

Description	Quantity
Powder Pillow Test:	
PhosVer <sup>®</sup> 3 Phosphate Reagent powder pillow	1
Sample Cells, 1-inch, 10-mL	2
Stopper for 18 mm Tube (square sample cells only)	1
AccuVac Test:	
Collect at least 40 mL of sample in a 50-mL beaker	40 mL
PhosVer <sup>®</sup> 3 Phosphate Reagent AccuVac <sup>®</sup> Ampul	1

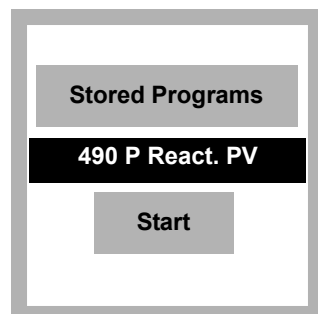
## Phosphorus, Reactive (Orthophosphate)

Collect the following items: (continued)

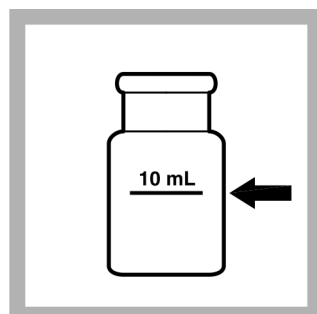
Description	Quantity
Beaker, 50-mL	1
Sample Cell, 10-mL round	1
Stopper for 18-mm Tube (supplied with PhosVer AccuVacs)	1

See [Consumables and replacement items](#) for reorder information.

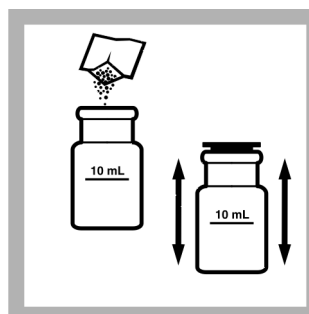
### PhosVer 3 (Ascorbic Acid) method for powder pillows



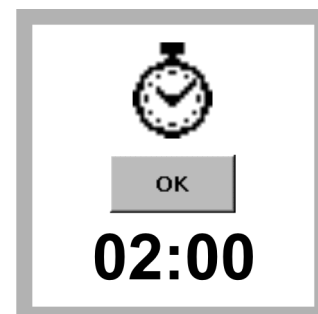
1. Select the test.  
Insert an adapter if required (see [Instrument-specific information](#)).



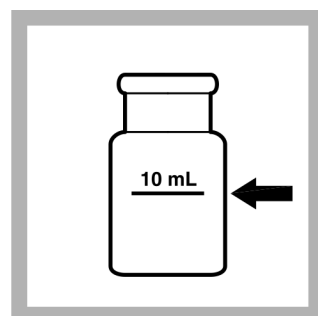
2. Fill a sample cell with 10-mL of sample.



3. **Prepared Sample:**  
Add the contents of one PhosVer 3 phosphate Powder Pillow to the cell. Immediately stopper and shake vigorously for 30 seconds.



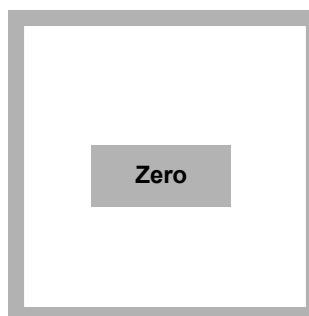
4. Start the instrument timer.  
A two-minute reaction period will begin. If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.



5. **Blank Preparation:**  
Fill a second sample cell with 10 mL of sample.



6. When the timer expires, wipe the blank and insert it into the cell holder.

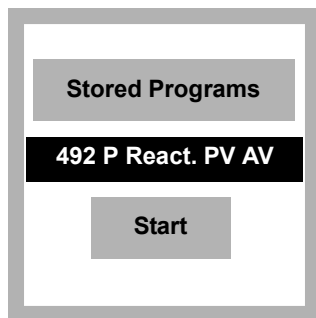


7. **ZERO** the instrument.  
The display will show:  
0.00 mg/L PO<sub>4</sub><sup>3-</sup>

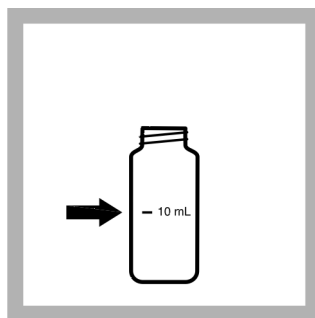


8. Wipe the prepared sample and insert it into the cell holder.  
**READ** the results in mg/L PO<sub>4</sub><sup>3-</sup>.

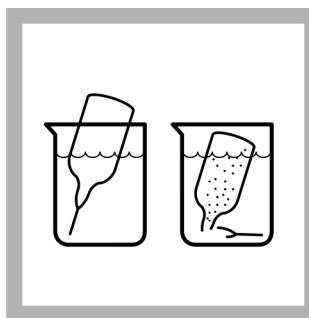
PhosVer 3 (Ascorbic Acid) method for AccuVac® Ampuls



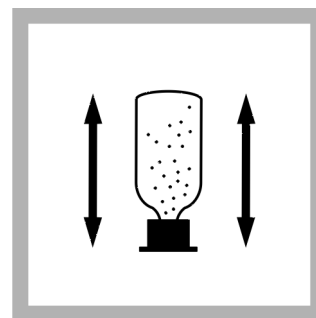
1. Select the test.  
Insert an adapter if required (see [Instrument-specific information](#)).  
Refer to the user manual for orientation.



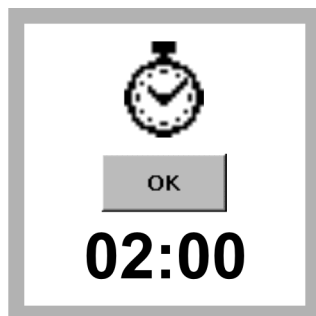
2. **Blank Preparation:**  
Fill a sample cell with 10-mL of sample.



3. **Prepared Sample:**  
Fill a PhosVer 3 Phosphate AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.



4. Secure an Ampul cap over the tip of the Ampul. Shake the Ampul for approximately 30 seconds.  
Accuracy is unaffected by undissolved powder.



5. Start the instrument timer.  
A two-minute reaction period will begin. If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.



6. When the timer expires, wipe the blank and insert it into the cell holder.  
**ZERO** the instrument.  
The display will show:  
0.00 mg/L PO<sub>4</sub><sup>3-</sup>



7. Wipe the prepared sample and insert it into the cell holder.  
**READ** the results in mg/L PO<sub>4</sub><sup>3-</sup>.

## Interferences

**Table 2 Interfering substances**

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level.
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Hydrogen Sulfide	Interferes at any level
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. pH 2–10 is recommended.
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Turbidity or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. For highly turbid or colored samples, add the contents of one Phosphate Pretreatment <sup>1</sup> Powder Pillow to 25 mL of sample. Mix well. Use this solution to zero the instrument.
Zinc	Greater than 80 mg/L

<sup>1</sup> See [Optional reagents and apparatus](#).

## Sample collection, preservation and storage

- Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution\* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.
- For best results, analyze samples immediately.
- If prompt analysis is not possible, preserve samples by filtering immediately and storing at 4 °C (39 °F) for up to 48 hours.
- Return the sample to room temperature before analysis.

## Accuracy check

### Standard additions method (sample spike)

Required for accuracy check:

- Phosphate 10-mL Ampule Standard, 50-mg/L PO<sub>4</sub><sup>3-</sup>
- Ampule breaker
- TenSette Pipet

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.

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\* See [Optional reagents and apparatus](#).

2. Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADDITIONS
DR 2800	OPTIONS>MORE>STANDARD ADDITIONS
DR 2700	OPTIONS>MORE>STANDARD ADDITIONS
DR/2500	OPTIONS>STANDARD ADDITIONS
DR/2400	OPTIONS>STANDARD ADDITIONS

3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Prepare a 0.1-mL sample spike by adding 0.1 mL of standard to the unspiked sample. Press the timer icon. After the timer expires, read the result.
6. Prepare a 0.2-mL sample spike by adding 0.1 mL of standard to the 0.1-mL sample spike. Press the timer icon. After the timer expires, read the result.
7. Prepare a 0.3-mL sample spike by adding 0.1 mL of standard to the 0.2-mL sample spike. Press the timer icon. After the timer expires, read the result. Each addition should reflect approximately 100% recovery.

**Standard additions method for AccuVac Ampuls (sample spike)**

Required for accuracy check:

- Mixing cylinders (3)
1. Fill three mixing cylinders each with 50-mL of sample and spike with 0.2 mL, 0.4 mL and 0.6 mL of standard.
  2. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
  3. Analyze each standard addition sample as described in the [PhosVer 3 \(Ascorbic Acid\) method for AccuVac® Ampuls](#).
  4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.

**Standard solution method**

*Note: Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate standard solution, 50 mg/L
  - Deionized water
  - 100-mL Class A volumetric flask
  - Class A volumetric pipet
  - TenSette Pipet
1. Prepare a 2.00 mg/L phosphate standard solution as follows:
    - a. Pipet 4.00 mL of Phosphate Standard, 50-mg/L, into a 100-mL volumetric flask.

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b. Dilute to volume with demineralized water. Mix well. Prepare this solution daily.

**Note:** Alternately, use one of the mixed parameter standards listed in [Recommended standards](#). These contain 2.0 mg/L phosphate.

- Use this solution in place of the sample. Follow the [PhosVer 3 \(Ascorbic Acid\) method for powder pillows](#) test procedure.
- To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADJUST
DR 2800	OPTIONS>MORE>STANDARD ADJUST
DR 2700	OPTIONS>MORE>STANDARD ADJUST
DR/2500	OPTIONS>STANDARD ADJUST
DR/2400	OPTIONS>STANDARD ADJUST

- Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

## Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—DConcentration per 0.010 DAbs
490	DR 5000	2.00 mg/L PO <sub>4</sub> <sup>3-</sup>	1.98–2.02 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>
	DR 2800	2.00 mg/L PO <sub>4</sub> <sup>3-</sup>	1.98–2.02 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>
	DR 2700	2.00 mg/L PO <sub>4</sub> <sup>3-</sup>	1.98–2.02 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>
	DR/2500	1.00 mg/L PO <sub>4</sub> <sup>3-</sup>	0.97–1.03 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>
	DR/2400	1.00 mg/L PO <sub>4</sub> <sup>3-</sup>	0.97–1.03 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
492	DR 5000	2.00 mg/L PO <sub>4</sub> <sup>3-</sup>	1.98–2.02 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>
	DR 2800	2.00 mg/L PO <sub>4</sub> <sup>3-</sup>	1.98–2.02 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>
	DR 2700	2.00 mg/L PO <sub>4</sub> <sup>3-</sup>	1.98–2.02 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>
	DR/2500	1.00 mg/L PO <sub>4</sub> <sup>3-</sup>	0.98–1.02 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>
	DR/2400	1.00 mg/L PO <sub>4</sub> <sup>3-</sup>	0.98–1.02 mg/L PO <sub>4</sub> <sup>3-</sup>	0.02 mg/L PO <sub>4</sub> <sup>3-</sup>

## Summary of method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm

## Consumables and replacement items

### Required reagents

Description	Quantity/Test	Unit	Catalog number
PhosVer® 3 Phosphate Reagent Powder Pillows, 10-mL	1	100/pkg	2106069
OR			
PhosVer® 3 Phosphate Reagent AccuVac® Ampuls	1	25/pkg	2508025

### Required apparatus (powder pillows)

Description	Quantity/Test	Unit	Catalog number
Stopper for 18 mm Tube	1	6/pkg	173106

### Required apparatus (AccuVac)

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Stopper for 18 mm Tube	1	6/pkg	173106

### Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 10-mL Voluette® Ampul, 50-mg/L as PO <sub>4</sub>	16/pkg	17110
Phosphate Standard Solution, 50-mg/L as PO <sub>4</sub>	500 mL	17149
Phosphate Standard Solution, 1-mg/L as PO <sub>4</sub>	500 mL	256949
Standard, Drinking Water, Mixed Parameter, Inorganic: F, NO <sub>3</sub> , PO <sub>4</sub> , SO <sub>4</sub>	500 mL	2833049
Wastewater Effluent Standard, for mixed parameters: NH <sub>3</sub> -N, NO <sub>3</sub> -N, PO <sub>4</sub> , COD, SO <sub>4</sub> , TOC	500 mL	2833249
Water, deionized	4 L	27256

### Optional reagents and apparatus

Description	Unit	Catalog number
Hydrochloric Acid Solution, 6.0N, 1:1	500 mL	88449
Mixing Cylinder 50 mL	each	189641
Phosphate Treatment Powder Pillow	100/pkg	1450199
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001 <sup>1</sup>	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001 <sup>1</sup>	1000/pkg	2185628
Pipet, TenSette, Pipet, 1.0 - 10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970010 <sup>1</sup>	50/pkg	2199796
Pipet Tips, for TenSette Pipet 1970010 <sup>1</sup>	250/pkg	2199725
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

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### Optional reagents and apparatus

Description	Unit	Catalog number
pH Paper, 0–14 pH range	100/pkg	2601300
AccuVac snapper	each	2405200
AccuVac ampule blanks	25/pkg	2677925
Flask, volumetric, 100 mL	each	1457442
Pipet, volumetric, Class A, 4 mL	each	1451504
AccuVac ampule drainer	each	4103600

<sup>1</sup> Other sizes are available

### Optional standards

Description	Unit	Catalog number
Voluette Ampule breaker 10 mL	each	2196800
Phosphate, 10 mg/L	946 mL	1420416
Phosphate, 15 mg/L	100 mL	1424342
Phosphate; 100 mg/L	100 mL	1436832
Phosphate; 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210
Phosphate; 500 mg/L	100 mL	1424232



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