

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01113

Amino Acid Method¹**0.23 to 30.00 mg/L PO₄³⁻****Method 8178****Reagent Solution****Scope and application:** For water, wastewater and seawater.¹ Adapted from Standard Methods for the Examination of Water and Wastewater.



Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows sample cell and orientation requirements for reagent addition tests, such as powder pillow or bulk reagent tests.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information

Instrument	Sample cell orientation	Sample cell
DR 6000 DR 3800 DR 2800 DR 2700 DR 1900	The fill line is to the right.	2495402 
DR 5000 DR 3900	The fill line is toward the user.	
DR 900	The orientation mark is toward the user.	2401906 

Before starting

Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.

For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option.

The contents of one Amino Acid Reagent Powder Pillow can be used as an alternative to the 1 mL of Amino Acid Reagent Solution in the test procedure.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

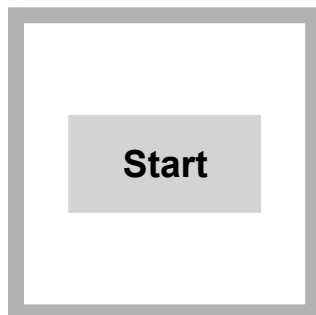
Description	Quantity
Amino Acid Reagent	1 mL
Cylinder, 25-mL, graduated mixing	1
Molybdate Reagent	1 mL
Sample cells (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	2

Refer to [Consumables and replacement items](#) on page 5 for order information.

Sample collection and storage

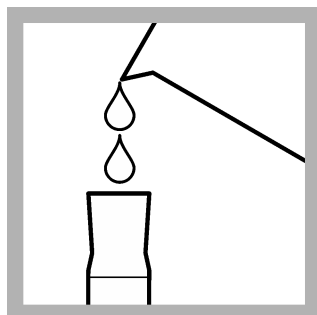
- Collect samples in clean glass or plastic bottles that have been cleaned with 6 N (1:1) hydrochloric acid and rinsed with deionized water.
- Do not use a detergent that contains phosphate to clean the sample bottles. The phosphate in the detergent will contaminate the sample.
- Analyze the samples as soon as possible for best results.
- If immediate analysis is not possible, immediately filter and keep the samples at or below 6 °C (43 °F) for a maximum of 48 hours.
- Let the sample temperature increase to room temperature before analysis.

Powder pillow procedure

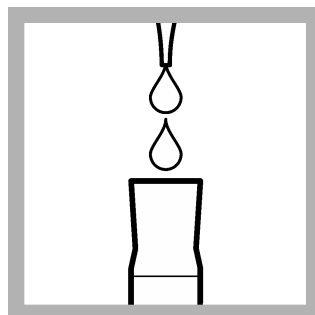


1. Start program **485 P React. Amino**. For information about sample cells, adapters or light shields, refer to [Instrument-specific information](#) on page 1.

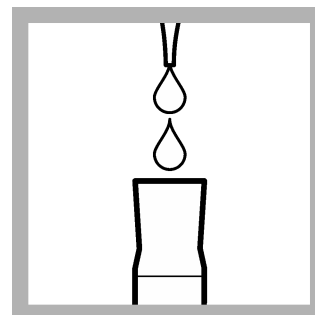
Note: Although the program name can be different between instruments, the program number does not change.



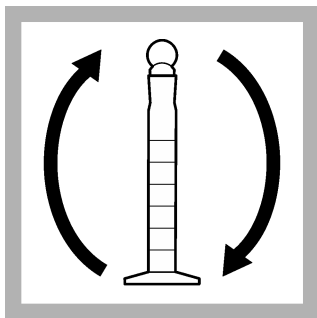
2. **Prepare the sample:** Fill a mixing cylinder to the 25-mL line with sample.



3. **Prepare the sample:** Add 1 mL of Molybdate Reagent.



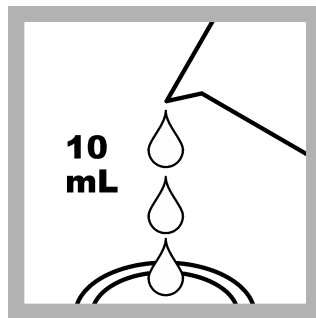
4. Add 1 mL of Amino Acid Reagent Solution.



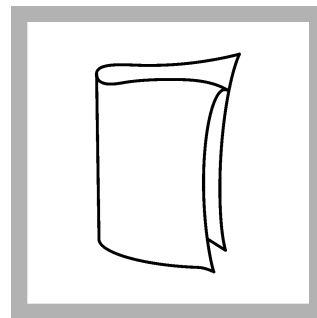
5. Put the stopper on the mixing cylinder. Invert the mixing cylinder several times to mix. A blue color shows if phosphate is present in the sample.



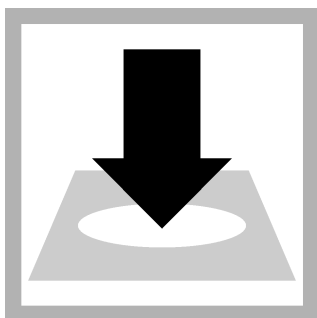
6. Start the instrument timer. A 10-minute reaction time starts. Prepare the blank while the timer is counting down.



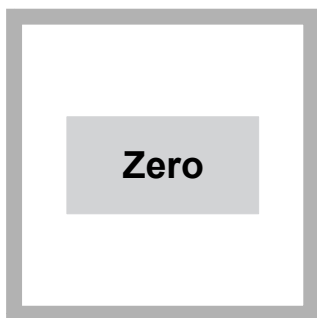
7. **Prepare the blank:** Fill a sample cell with 10 mL of untreated sample.



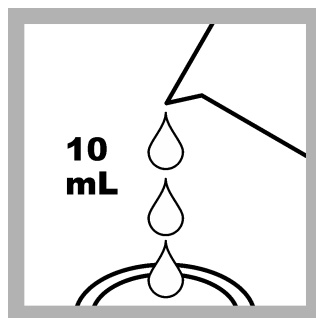
8. When the timer expires, clean the blank sample cell.



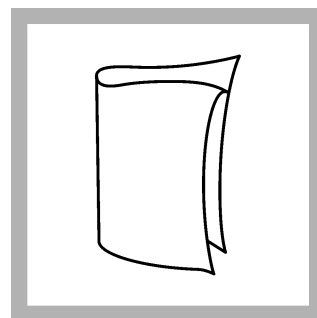
9. Insert the blank into the cell holder.



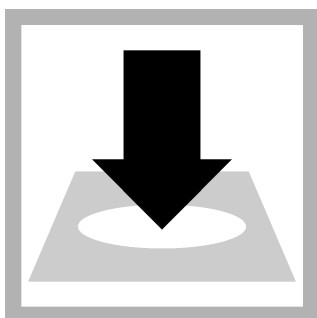
10. Push **ZERO**. The display shows 0.00 mg/L PO_4^{3-} .



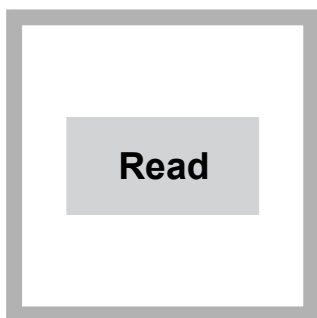
11. Fill a second sample cell with 10-mL of the prepared sample.



12. Clean the prepared sample cell.



13. Insert the prepared sample into the cell holder.



14. Push **READ**. Results show in mg/L PO_4^{3-} .

Interferences

Interfering substance	Interference level
Calcium	More than 10,000 mg/L as CaCO_3
Chloride	More than 150,000 mg/L Cl^-
Colored samples	Add 1 mL of 10 N Sulfuric Acid Standard Solution to another 25-mL sample. Use this instead of untreated sample as the blank to zero the instrument. Use a pipet and pipet filler to measure the sulfuric acid standard.
High salt levels (Na^+)	May cause low results. To eliminate this interference, dilute the sample until two successive dilutions give about the same result.

Interfering substance	Interference level
Magnesium	More than 40,000 mg/L as CaCO ₃
Nitrite (NO ₂ ⁻)	Bleaches the blue color. Remove nitrite interference by adding 0.10 g of sulfamic acid to 50 mL sample. Swirl to mix. Use this treated sample in the test procedure.
Phosphates, high levels (PO ₄ ³⁻)	As the concentration of phosphate increases, the color changes from blue to green, then to yellow and finally to brown. The brown color may suggest a concentration as high as 100,000 mg/L PO ₄ ³⁻ . If a color other than blue is formed, dilute the sample and retest.
Sulfide (S ²⁻)	Sulfide interferes. For samples with a sulfide concentration less than 5 mg/L, remove sulfide interference as follows: <ol style="list-style-type: none"> 1. Add 50 mL of sample to an Erlenmeyer flask. 2. Add Bromine Water by drops with constant swirling until a permanent yellow color develops. 3. Add Phenol Solution by drops until the yellow color just disappears. Use this treated sample in the test procedure.
Temperature	For best results, sample temperature should be 21 ± 3 °C (70 ± 5 °F).
Turbidity	May give inconsistent results for two reasons. Some suspended particles may dissolve because of the acid used in the test. Also, desorption of orthophosphate from particles may occur. For highly turbid samples, add 1 mL of 10 N Sulfuric Acid Standard Solution to another 25-mL sample. Use this instead of untreated sample as the blank to zero the instrument. Use a pipet and pipet filler to measure the sulfuric acid standard.
Highly buffered samples or extreme sample pH	Can prevent the correct pH adjustment of the sample by the reagents. Sample pre-treatment may be necessary.

Accuracy check

Standard additions method (sample spike)

Use the standard additions method (for applicable instruments) to validate the test procedure, reagents and instrument and to find if there is an interference in the sample.

Items to collect:

- Phosphate 2-mL Ampule Standard, 500-mg/L PO₄³⁻
 - Ampule breaker
 - Pipet, TenSette®, 0.1–1.0 mL and tips
 - Mixing cylinders, 25-mL (3)
1. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
 2. Go to the Standard Additions option in the instrument menu.
 3. Select the values for standard concentration, sample volume and spike volumes.
 4. Open the standard solution.
 5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard solution, respectively, to three 25-mL portions of fresh sample. Mix well.
 6. Use the test procedure to measure the concentration of each of the spiked samples. Start with the smallest sample spike. Measure each of the spiked samples in the instrument.
 7. Select **Graph** to compare the expected results to the actual results.

Note: If the actual results are significantly different from the expected results, make sure that the sample volumes and sample spikes are measured accurately. The sample volumes and sample spikes that are used should agree with the selections in the standard additions menu. If the results are not within acceptable limits, the sample may contain an interference.

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- 10-mg/L Phosphate Standard Solution

1. Use the test procedure to measure the concentration of the standard solution.
2. Compare the expected result to the actual result.

Note: The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are slight variations in the reagents or instruments.

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% confidence interval)	Sensitivity Concentration change per 0.010 Abs change
485	10.00 mg/L PO ₄ ³⁻	9.86–10.14 mg/L PO ₄ ³⁻	0.20 mg/L PO ₄ ³⁻

Summary of method

In a highly acidic solution, ammonium molybdate reacts with orthophosphate to form molybdophosphoric acid. This complex is then reduced by the amino acid reagent to yield an intensely colored molybdenum blue compound. The measurement wavelength is 530 nm for spectrophotometers or 520 nm for colorimeters.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
High Range Reactive Phosphorus Reagent Set	—	100 tests	2244100
Includes:			
Amino Acid Reagent	1 mL	100 mL MDB	193432
Molybdate Reagent	1 mL	100 mL MDB	223632

Required apparatus

Description	Quantity/test	Unit	Item no.
Mixing cylinder, graduated, 25-mL, glass stopper	1	each	189640

Recommended standards and apparatus

Description	Unit	Item no.
Phosphate Standard Solution, 10-mg/L as PO ₄	946 mL	1420416
Phosphate Standard Solution, 2-mL PourRite [®] Ampule, 500-mg/L PO ₄ ³⁻	16/pkg	1424220
Wastewater Effluent Standard Solution, Mixed Parameter, for NH ₃ -N, NO ₃ -N, PO ₄ ³⁻ , COD, SO ₄ ²⁻ , TOC	500 mL	2833249
Wastewater Influent Standard Solution, Mixed Parameter, for NH ₃ -N, NO ₃ -N, PO ₄ , COD, SO ₄ , TOC	500 mL	2833149

Recommended standards and apparatus (continued)

Description	Unit	Item no.
Water, deionized	4 L	27256
Ampule Breaker, 2-mL PourRite® Ampules	each	2484600

Optional reagents and apparatus

Description	Unit	Item no.
Amino Acid Reagent Powder Pillows	100/pkg	80499
Bromine Water, 30 g/L	29 mL	221120
Flask, Erlenmeyer, 125-mL	each	50543
Hydrochloric Acid Solution, 6.0 N (1:1)	500 mL	88449
Phenol Solution, 30-g/L	29 mL	211220
Sulfamic Acid, 454 g	each	234401
Sulfuric Acid Standard Solution, 10 N	1000 mL	93153
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Paper, pH, 0–14 pH range	100/pkg	2601300
Filter paper, folded, 3–5-micron, 12.5-cm	100/pkg	69257
Funnel, poly, 65-mm	each	108367
Thermometer, non-mercury, –10 to +225 °C	each	2635700
Bottle, sampling, with cap, low density polyethylene, 250-mL	12/pkg	2087076

Optional standards

Description	Unit	Item no.
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800
Phosphate Standard Solution, 3-mg/L as PO ₄ ³⁻	946 mL	2059716
Phosphate Standard Solution, 15-mg/L as PO ₄ ³⁻	100 mL	1424342
Phosphate Standard Solution, 30-mg/L as PO ₄ ³⁻	946 mL	1436716
Phosphate Standard Solution, 50-mg/L, 10-mL Voluette® Ampules	16/pkg	17110
Phosphate Standard Solution, 100-mg/L as PO ₄	100 mL	1436832
Phosphate Standard Solution, 10-mL ampule, 500 mg/L as PO ₄	16/pkg	1424210
Phosphate Standard Solution, 500-mg/L as PO ₄	100 mL	1424232



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