METTLER TOLEDO iNORGANIC VENTURES

Colorimetric Method for Measuring Phosphorus as Orthophosphate

ASCORBIC ACID METHOD (ISO 15923-1)

Method Description

Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

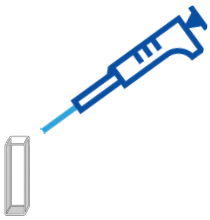
Test Procedure

1. Pipette 3450 µL* of the sample into the cuvette.

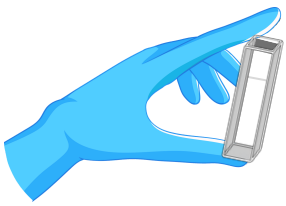
** µL = microliter*

2. Pipette 390 µL of **RGT-PHOSPHATE-PO4-BTL-1-OF-2** into the cuvette.

3. Pipette 170 µL of **RGT-PHOSPHATE-PO4-BTL-2-OF-2** into the cuvette.



4. Cap cuvette, and shake 10-15 times to mix reagent & sample within.



5. Wait duration of reaction time: 10 minutes. **Note:** Solution should turn blue during reaction time.

6. Insert cuvette into the UV-Vis and record absorbance of sample.

Be sure to use a new pipette tip for every addition. Replace the pipette tip between every step. Always dispose of all waste properly.

Expected Limits of Detection

Path Length (mm)	Method Detection Limit (mg P/L)
10	0.010
50	0.004

Detection limits were calculated to be equal to three times the standard deviation of a series of 10 replicate measurements of the calibration blank.

Preparation of Calibration Standards

Use the following volumes to prepare a Calibration Curve using a stock standard containing 10 mg/L of phosphorus from orthophosphate. Select at least 3-4 calibration standards that bracket your expected concentration range. Dilute all standards in DI H2O or a matrix most suited to your sample type.

Concentration (mg P/L)	Volume of Standard Aliquot (mL)	Final Volume (mL)
0.00	0.00	50
0.02	0.20	**100***
0.05	0.25	50
0.10	0.50	50
0.25	1.25	50
0.50	2.50	50
0.65	3.25	50
0.75	3.75	50
0.85	4.25	50
1.00	5.00	50

** Larger final volume needed for 0.20 mg P/L standard due to small standard aliquot volume.*

Calibration Tip:

If using different calibration standards to construct your calibration curve, ensure that the concentrations of neighboring standards do not exceed a ten fold difference.