**1.0 Procedure title**

Measuring Phosphate of Algae Culture using acid-molybdate in 96-well Plates


**2.0 Procedure impacts and concerns**

**2.1 Safety**

The reagents used in this assay are potentially harmful to human health. Especially important are the use of chemical resistant gloves, and safety glasses for eye protection.

Review the MSDS for each of the reagents used before starting the assay.

The dilution of the concentrated sulfuric acid must be performed in a chemical fume hood.

Ammonium molybdate(VI) tetrahydrate is a Confirmed Animal Carcinogen with Unknown Relevance to Humans (listed as 'Molybdenum soluble compounds'). It is also listed as causing mutation in microorganisms.

Antimony Potassium Tartrate Trihydrate : Target Organs: Kidneys, heart, liver. Oral, rat: LD50 = 115 mg/kg

Sodium Dodecyl Sulfate : Target Organs: Respiratory system, Skin, Eyes. Dermal, rabbit: LD50 = 580 mg/kg

**2.2 Quality**

Use ultra pure water for diluting samples and making reagents to obtain the most accurate results.

Wiping the pipette tip before delivering small volumes, < 10 ul, was essential for accuracy, as verified by the validation.

The effect of reading the color developed plate after 30 minutes increases the variances between replicates. To assure valid results read the plate before 30 minutes of color development.

When preparing stock solutions do not use glassware that has been cleaned with a detergent as residues will interfere with results. Only use glassware that has been acid washed and rinsed with milli-Q water.

**2.3 Delivery**

N/A

**2.4 Environmental**

All waste and unused reagents must be collected in a common container for professional disposal.

Antimony Potassium Tartrate Trihydrate: Hazardous to aquatic life.

Sodium Dodecyl Sulfate: Hazardous to aquatic life.

**2.5 Cost**

The most expensive reagent per experiment was the Gibco Ultra Pure water. Making only the minimum volume of reagents for the required workload will decrease the cost of performing this assay by minimizing waste.

**2.6 Compliance**

Ammonium molybdate(VI) tetrahydrate, CAS# 12054-85-2, can be found on the California right to know lists - listed as Molybdenum compounds, n.o.s..

Use of the Water Chemistry Template Macro helps to ensure valid data results, as well as expedite the analysis process.

**3.0 Related Procedures & Documents**

Filtration of algae culture for analytical methods
Use of the plate reader for Absorbance Endpoint
Using the Water Chemistry Template Macro (??)
Operator instructions and manuals for all equipment used while performing the procedure.
MSDS for all reagents used while performing the procedure.

**4.0 Responsibilities**

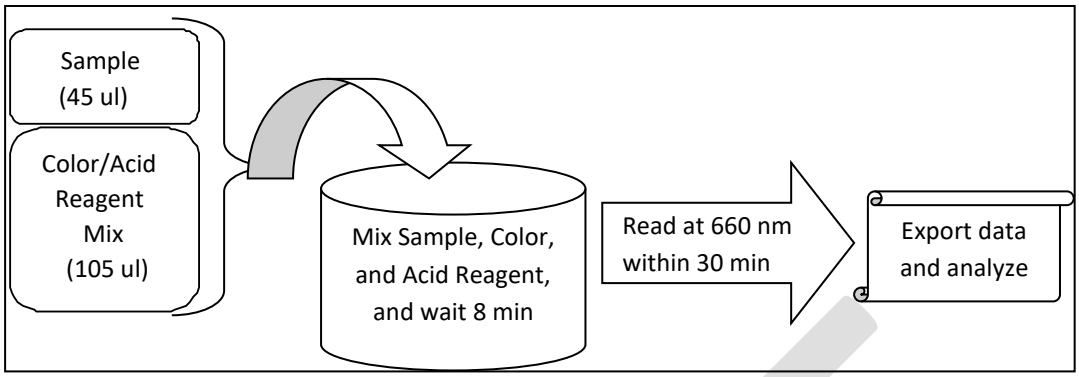
| | | |
|---|---|---|
| Document Owner | Manage content and distribution | Jeremy Ferrara |
| Process Owner | Responsible for content and process validation | Jeremy Ferrara |
| Plant Manager | Responsible for implementation and conformance | Nicole Heaps |

**5.0 Process**

**5.1 Process description**

The phosphate 96-well assay was based on Seal Analytical AQ1 method o-Phosphate-P in Drinking, Saline and Surface Waters, and Domestic and Industrial Wastes. The method has been summarized by Sapphire Team in file SES00001, SOP Title Phosphate Detection Assay. Ammonium molybdate and antimony potassium tartrate react in acid media with o-phosphate to form an antimony-phosphomolybdate complex. This complex is reduced to a blue-colored complex by ascorbic acid. Color intensity is proportional to the phosphorus concentration. The procedure can be simplified to mixing a sample diluted in water with two reagents: 1) the Color Reagent, and 2) the Acid reagent. The assay is documented to be useful for analyte concentration between 0.125 to 12.5 mg P/L, estimated reporting level 0.1 mg P/L. Note that the symbol "P" refers to *phosphorous*. It is important to differentiate this from the meaning of *phosphate,* referring to the chemical species $PO_4^{3-}$. The actual species used to create standards is *Potassium dihydrogen orthophosphate, anhydrous*; formulae $KH_2PO_4$. The following table clarifies the relationship between expected response boundaries to be tested.

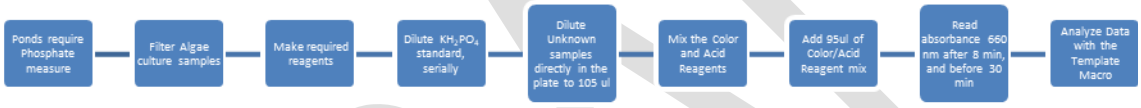
| Species | FW | Low Range mg/L | High Range mg/L |
|---|---|---|---|
| Potassium dihydrogen orthophosphate, anhydrous | 136.09 | 0.44 | 65.91 |
| Phosphate, (o-phosphate, $PO4^{3-}$) | 94.968 | 0.31 | 46.00 |
| P (phosphorous) | 30.97 | 0.1 | 15 |




The schematic shown the figure can be accomplished by using a final volume of 200 ul. Cost of reagents per plate was about $0.47. The entire assay can be completed for two plates within 1 hour 15 minutes.

The average time per replicate was about 0.5 minutes.

### 5.2 Process diagram




### 5.3 Equipment

Vortexer
15 ml PP Conical Tube (Fisher Cat# 05-539-12)
1 L, 500 ml and 150 ml Filter Unit, 0.22 um Cellulose Nitrate (Fisher Cat# 09-761-141, 09-761-102, 09-761-119)
Magnetic stir plate and stir bar
150 ml, 500 ml, and 1L Beaker
Pipette aid
Pipette and Pipette tips (20, 200, and 1000 ul)
Pipettes (5, 10, 25 ml)
1.5 ml micro centrifuge tubes (Fisher Cat# 05-408-129)
Clear 96-well culture plates (BD Falcon Cat# 353072)
Kimwipes (Fisher Cat# 06-666-11C)
Spectrophotometer (Abs 520 nm) to read 96-well plates, Molecular Diagnostics SpectraMax M2

**5.4 Reagents**

Gibco Ultra Pure Distilled H20 (Invitrogen Cat# 10977-015)
Sulfuric Acid (12N) (NFPA 3,0,2) (Fisher Cat# 320501)
Ammonium Molybdate (NFPA 2,0,0) (Sigma Cat# M1019)
Antimony Potassium Tartrate Trihydrate (3g/L) (NFPA 2,0,0) (Fisher Cat# A867-250)
Sodium Dodecyl Sulfate (NFPA 2,3,0) (Fisher Cat# BP166-500)
Potassium dihydrogen phosphate, Anhydrous (NFPA 1,0,0) (Sigma Cat# 60216)
L - Ascorbic Acid (NFPA 2,1,0) (Fisher Cat# A5960)
Stock solution at 50 ppm P (RICCA chemical Cat# 5830-4)

**5.5 Process steps**

5.5.1 Preparation an algae culture filtrate.

i. Refer to procedure POND0001, Filtration of algae culture for analytical methods. Ref: TBD

5.5.2 Prepare serial dilutions of calibration standard in microcentrifuge tubes.

Each 96-well assay plate used in the experiment must include Blanks, Standards, and QC Check standards in Row A and Row B. The stock of 1000 ppm P (153 ppm $PO_4^{3-}$) is to be diluted first to a 50 ppm P stock solution that can be stored at 4°C for one month. The 50 ppm P stock solution is then diluted to form an 8 point calibration curve spanning the working range of the assay, between 1 ppm P and 12.5 ppm P/L. The dilution table is listed below.

| Cal# | Target Conc (ppm) | Source Conc (ppm P) | Dilution (1:x) | Conc (ppm $PO_4^{3-}$) | Method |
|---|---|---|---|---|---|
| 1 | 12.5 | 50.0 | 4.00 | 38.33 | Add 250 ul of Source to 750 ul of |
| 2 | 10.5 | 50.0 | 4.76 | 32.2 | Add 210 ul of Source to 790 ul of |
| 3 | 8.5 | 50.0 | 5.88 | 26.1 | Add 170 ul of Source to 830 ul of |
| 4 | 7.0 | 50.0 | 7.14 | 21.5 | Add 140 ul of Source to 860 ul of |
| 5 | 5.5 | 50.0 | 9.09 | 16.9 | Add 110 ul of Source to 890 ul of |
| 6 | 4.0 | 50.0 | 12.50 | 12.3 | Add 80 ul of Source to 920 ul of |
| 7 | 2.5 | 50.0 | 20.00 | 7.7 | Add 50 ul of Source to 950 ul of |
| 8 | 1.0 | 50.0 | 50.00 | 3.1 | Add 20 ul of Source to 980 ul of |

5.5.3 Prepare the QC check standards – Hi and Lo

Dilute the RICCA 50 ppm P standard in GUH in bulk using a 50 mL volumetric flask.

| Target Conc (ppm of P) | Source (ppm) | DF | Source (mL) | GUH (mL) |
|---|---|---|---|---|
| 5.0 | 50.0 | 10 | 5.0 | 45.0 |
| 1.5 | 50.0 | 33.33 | 1.5 | 48.5 |

5.5.3 Deliver GUH diluent Blank and Media Source to A1/B1 and A2/B2, respectively.

i. Add 45 uL of GUH diluent to wells A1 and B1 for a quality control check for contaminated water source.
ii. Add 45 uL of the media source used in the algae culture to wells A2 and B2 to serve as an additional quality control check.

Plate Map

| | Col1 | Col2 | Col3 | Col4 | Col5 | Col6 | Col7 | Col8 | Col9 | Col10 | Col11 | Col12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | GUH dil | Media | Cal1 | Cal2 | Cal3 | Cal4 | Cal5 | Cal6 | Cal7 | Cal8 | QC Lo | QC Hi |
| B | GUH dil | Media | Cal1 | Cal2 | Cal3 | Cal4 | Cal5 | Cal6 | Cal7 | Cal8 | QC Lo | QC Hi |
| C | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 |
| D | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 |
| E | S13 | S14 | S15 | S16 | S17 | S18 | S19 | S20 | S21 | S22 | S23 | S24 |
| F | S13 | S14 | S15 | S16 | S17 | S18 | S19 | S20 | S21 | S22 | S23 | S24 |
| G | S25 | S26 | S27 | S28 | S29 | S30 | S31 | S32 | S33 | S34 | S35 | S36 |
| H | S25 | S26 | S27 | S28 | S29 | S30 | S31 | S32 | S33 | S34 | S35 | S36 |

Reference the Plate Map during the next steps.

5.5.4 Deliver standards to Row A and Row B.

i. Add 45 uL of each of the 8 calibration standards to Row A in decreasing concentration starting with Well A1 at 12.5 ppm.

Dilute standards in microcentrifuge tubes.

5.5.5 Deliver QC Lo and QC Hi check standards.

i. Add 45 uL of QC Lo check standard to wells A11 and B11 for data validation.
ii. Add 45 uL of QC Hi check standard to A12 and B12 for data validation.

5.5.6 Dilute samples in 2 mL deep well plates.

Dilute the unknowns 1:4 (Add 100 uL of sample to 300 uL of GUH) in a deep well plate. Mix thoroughly using a plate shaker. Add 45 uL directly to the 96-well plate.

5.5.7 Prepare the phosphate color master mix.

i. Mix reagents as described in the table below.
ii. Cap and vortex to mix.

| | DF | 1 plate | 2 plates | 3 plates |
|---|---|---|---|---|
| GUH (mL) | 2.58 | 5.78 | 11.74 | 17.52 |
| Working Ascorbic Acid (mL) | 3.88 | 3.84 | 7.81 | 11.65 |
| Working PO4 Color Reagent (mL) | 2.82 | 5.28 | 10.74 | 16.03 |
| Total (mL) | | 14.9 | 30.3 | 45.2 |

The volumes presented in the table provide enough Color reagent added to the full plates with approximately 0.5 mL dead volume.

5.5.8 Add 155 uL of Phosphate Color/Acid Reagent mix to all wells.

i. Use a multichannel pipette to deliver 155 uL of Phosphate color master mix to all wells.
ii. Pop any bubbles that form using a P-1000 multichannel pipette.
iii. Align the pipette tips over the bubbles in wells. Do not contact the liquid.
iv. Blow air through the pipette to pip the bubbles. Be careful not to cause splashing of the liquid.

Ensure the tips form a good seal before pipetting. Bubbles will cause error due to light scatter.

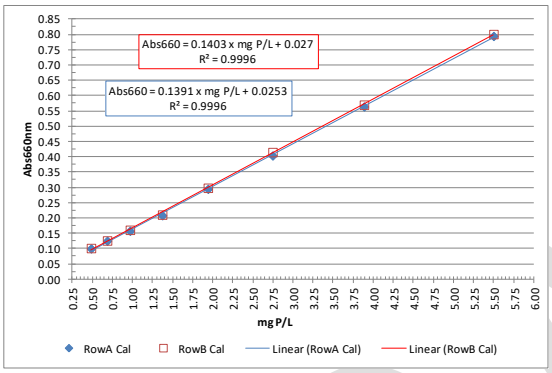
5.5.10 Measure Absorbance 660 nm after 8 min and before 30 min of color development.

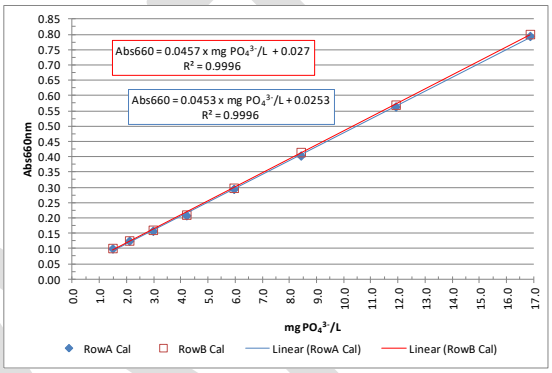
i. Measure the absorbance.
ii. Export data to text file.

5.5.11 Workup the data using the Water Chemistry Template Macro.

## 5.6 Example Data

5.6.1 Example of Calibration Standard Curve




5.6.2 Example of QC check standard results with Upper and Lower Bound at +/-3s, respectively.

The QC check standards are used to validate that the experiment was performed accurately using quality reagents. QC check standard results must be "within spec", between the Upper and Lower Bound determined during quality control studies.

| | QC lo | | QC hi | |
|---|---|---|---|---|
| | ppm P | ppm PO4 | ppm P | ppm PO4 |
| **Target** | 1.5 | 4.60 | 5.00 | 15.30 |
| **Mean** | n/a | n/a | 4.88 | 14.97 |
| **Std Dev** | n/a | n/a | 0.23 | 0.71 |
| **3 SD** | n/a | n/a | 0.70 | 2.14 |
| **Upper Bound** | 1.80 | 5.52 | 5.70 | 17.44 |
| **Lower Bound** | 1.30 | 3.68 | 4.30 | 13.16 |
| **%RSD** | n/a | n/a | 4.8% | 4.8% |
| **%DIF** | n/a | n/a | -2.4% | -2.2% |

**Note: Upper and lower bounds were set at the default +/- 20%. Historical data will be used to define the QC acceptance criteria once a statically significant number of QC values have been collected.**

**5.7 Preparation of Reagent Solutions**

Use reagent-grade chemicals, certified for analytical or general laboratory use. Use gibco ultra pure grade water that is free from organic contamination. Use glassware that has been acid washed and rinsed with nanopure water. Contamination of glassware by detergents will cause interference with the colorimetric chemistry.

5.7.1 SODIUM DODECYL SULFATE (10 w/v%)

10% SDS is also commercially available.

| | |
|---|---|
| Sodium dodecylsulfate | 50 g |
| GUH water | Dilute to 500 mL |

i. To a 500 mL volumetric flask add 50 g sodium dodecyl sulfate. Use 98.5% or better reagent grade, suitable for electrophoresis or molecular biology (e.g. Sigma Aldrich product numbers L-3771, L-4390 or L-4509).
ii. Bring the volume up to approximately 400 mL with GUH.
iii. Swirl or stir on a magnetic stir plate to dissolve.
iv. Dilute to 500 mL with GUH and mix gently.
v. Filter sterilize into a 500 ml filter unit (Fisher Cat# 09-761-102).
vi. Store at room temperature.

5.7.2 AMMONIUM MOLYBDATE TETRAHYDRATE (4 w/v%)

| | |
|---|---|
| Ammonium molybdate tetrahydrate | 4 g |
| GUH water | Dilute to 100 mL |

i. Add 4 g ammonium molybdate tetrahydrate and a magnetic stir bar to a 100 mL volumetric flask.
ii. Bring volume up to approximately 80 mL with GUH.
iii. Stir for at least 1 hour on a magnetic stir plate to dissolve.
iv. Dilute to 100 mL with GUH.
v. Filter sterilize into a 150 ml filter unit (Fisher Cat# 09-761-119).
vi. Store in a plastic bottle at 4$^{o}$C. For up to 1 month. Discard if it becomes blue or turbid.

5.7.3 SULFURIC ACID (5 N)

| | |
|---|---|
| Sulfuric Acid, concentrated | 70 mL |
| GUH water | Dilute to 500 mL |

**Note: Never add water to concentrated acid. Always slowly add concentrated acid to water.**

i. Perform this step in a chemical fume hood.
ii. Place about 400 ml of GUH into a 500 mL volumetric flask.
iii. Slowly add 70 mL concentrated Sulfuric Acid (37 N).
iv. Mix by gently swirling.
v. When cool to touch, bring volume to 500 mL with GUH.
vi. Transfer to a sealed glass bottle.
vii. Store in acid cabinet.

5.7.4 ANTIMONY POTASSIUM TARTRATE (3 g/L)

| | |
|---|---|
| Antimony potassium tartrate trihydrate | 1.5 g |
| GUH water | Dilute to 500 mL |

i. Dissolve 1.5 g antimony potassium tartrate trihydrate in about 400 mL GUH water in a 500 mL volumetric flask.
ii. Mix until reagent is completely dissolved.
iii. Dilute to 500 mL.
iv. Filter sterilize into a 500 ml filter unit (Fisher Cat# 09-761-102).
v. Cover bottle with Aluminum foil.
vi. Store at 4$^{o}$C in the dark for up to one month.

5.7.5 WORKING ASCORBIC ACID REAGENT, 8 g/L [with o-phosphate spike ]

| | |
|---|---|
| Ascorbic acid, fine granular | 0.80 g |
| Phosphate stock standard solution | 1000 ppm |
| Sodium dodecyl sulfate, 10% (w/v) above | 0.5 mL |
| GUH | Dilute to 100 mL |

i. Dissolve 0.8 g ascorbic acid in about 80 mL of GUH water.
ii. Spike this reagent with o-phosphate by delivering 10 uL of the phosphate stock standard solution (1000 mg P/L).
iii. Add 0.5 mL Sodium dodecyl sulfate (10 %).
iv. Dilute to 100 mL with GUH water and mix well.
v. Filter sterilize into a 150 ml filter unit (Fisher Cat# 09-761-119).
vi. Store at 4$^{o}$C for up to one week.
vii. Discard if the solution becomes yellowed.

5.7.6 WORKING PHOSPHATE COLOR REAGENT

| | |
|---|---|
| Sulfuric Acid, 5N | 45 mL |
| Ammonium molybdate, 4% w/v | 15 mL |
| Antimony potassium tartrate, 3 g/L | 8 mL |
| GUH water | Dilute to 100 mL |

i. To a 100 mL volumetric flask, add approximately 25 mL GUH water.
ii. Add 15 mL Ammonium Molybdate (4 w/v%).
iii. Add 8 mL Antimony Potassium Tartrate (3 g/l).
iv. Slowly add 45 mL Sulfuric Acid (5 N).
v. Swirl gently to mix.
vi. Dilute to 100 mL with GUH water.
vii. Filter sterilize into 150 ml filter unit (Fisher Cat# 09-761-119).
viii. Store in a plastic container for up to 3 weeks.
ix. Discard if the reagent turns blue or becomes turbid.

5.7.7 PHOSPHATE STOCK STANDARD (1000 ppm P, 4394 mg $KH_2PO_4$/L)

(NFPA 2,3,0)

| | |
|---|---|
| Potassium dihydrogen orthophosphate, anhydrous | 4.394 g |
| GUH water | Dilute to 1L |

i. Add 750 ml of GUH to a 1 L volumetric flask.
ii. Add 4.394 g Potassium dihydrogen orthophosphate, anhydrous ($KH_2PO_4$) to the flask.
iii. Cap the flask, seal with parafilm and invert to mix until crystals are dissolved.
iv. Dilute to 1 L with GUH water in a volumetric flask.
v. Mix thoroughly by inversion for at least 2 min.
vi. Aliquot volumes as necessary.
vii. Store at 4°C.

5.7.8 PREPARATION OF BULK QC CHECK STANDARDS

| |
|---|
| 50 ppm P Stock Solution (RICCA Chemical Company Cat # 5380-4) |
| Gibco UltraPure Distilled Water (Gibco Cat # 10977-015) |

| Target Conc (ppm of P) | Source (ppm) | DF | Source (mL) | GUH (mL) |
|---|---|---|---|---|
| 5 | 50 | 10 | 5 | 45 |
| 1.5 | 50 | 50 | 1.5 | 48.5 |

i. Add the appropriate volume (see table above) of stock solution to a 50 mL volumetric flask.
ii. Dilute to 50 mL with GUH water.
iii. Cap and wrap top with parafilm.
iv. Mix thoroughly by inversion.
v. Aliquot volumes as necessary.
vi. Store at 4°C.

## 6.0 Waste and Safety

### 6.1 Disposal of reagents

i. All unused reagents and reagent waste is to be collected into a single waste container for professional removal offsite.
ii. Fluid in plates is to be removed and collected with other unused reagents and reagent waste.
iii. Tips should be completely expelled into the assay wells, or into waste collection. When the tips are clear of any fluid, they can be placed in the trash.

### 6.2 Required PPE

i. Gloves and safety glasses are to be worn for the entire duration of the experiment.

### 6.3 Potential Health Effect of Reagents

6.3.1 Potential Health Effects of Sulfuric Acid, $H_2SO_4$

Inhalation: May cause irritation of the respiratory tract with burning pain in the nose and throat, coughing, wheezing, shortness of breath and pulmonary edema. Causes chemical burns to the respiratory tract. Inhalation may be fatal as a result of spasm, inflammation, edema of the larynx and bronchi, chemical pneumonitis and pulmonary edema. Because its vapor pressure is negligible, it exists in the air only as a mist or spray. Exposure may impair lung function and cause mucostasis (reduced mucous clearance).
Ingestion: May cause severe and permanent damage to the digestive tract. Causes gastrointestinal tract burns.
Skin Contact: Causes skin burns. The severity of injury depends on the concentration of the solution and the duration of exposure.
Eye Contact: Causes severe eye burns. May cause irreversible eye injury. May cause blindness. May cause permanent corneal opacification. The severity of injury depends on the concentration of the solution and the duration of exposure.
Chronic Exposure: Prolonged or repeated skin contact may cause dermatitis. Prolonged or repeated inhalation may cause nosebleeds, nasal congestion, erosion of the teeth, perforation of the nasal septum, chest pain and bronchitis.
Prolonged or repeated eye contact may cause conjunctivitis. Effects may be delayed. Workers chronically exposed to sulfuric acid mists may show various lesions of the skin, tracheobronchitis, stomatitis, conjunctivitis, or gastritis. Occupational exposure to strong inorganic acid mists containing sulfuric acid is carcinogenic to humans.

6.3.2 Potential Health Effects of Antimony Potassium Tartrate Trihydrate, $C_8H_4K_2O_{12}Sb_2{\cdot}3H_2O$

Inhalation: May cause nausea, vomiting, abdominal pain, diarrhea, chest tightness, weakness, and delayed pulmonary edema. Antimony compounds may enter the body through the lungs. Causes irritation of the mucous membrane and upper respiratory tract.
Ingestion: Harmful if swallowed. May cause gastrointestinal irritation with nausea, vomiting and diarrhea.
Skin Contact: Causes skin irritation.
Eye Contact: Causes eye irritation. May cause chemical conjunctivitis.
Chronic Exposure: Prolonged or repeated skin contact may cause dermatitis. Effects may be delayed. Laboratory experiments have resulted in mutagenic effects. Prolonged exposure may cause liver, kidney, and heart damage.

6.3.3 Potential Health Effects of Ammonium Molybdate(VI) Tetrahydrate, $H_{24}Mo_7N_6O_{24} \cdot 4H_2O$

Inhalation: Causes respiratory tract irritation. May be harmful if inhaled. Exposure may cause blood abnormalities.
Ingestion: Harmful if swallowed. May cause irritation of the digestive tract. May cause liver and kidney damage. Molybdenum toxicity in ruminants is characterized by symptoms of copper deficiency.
Skin Contact: Causes skin irritation. May be harmful if absorbed through the skin.
Eye Contact: Causes eye irritation.
Chronic Exposure: No information found.

6.3.4 Potential Health Effects of Sodium Dodecyl Sulfate, $CH_3(CH_2)_{10}CH_2OSO_3Na$

Inhalation: Irritating to respiratory system. May be harmful if inhaled. May cause allergic respiratory reaction.
Ingestion: Harmful if swallowed. Causes gastrointestinal tract irritation.
Skin Contact: Irritating to skin. Harmful in contact with skin.
Eye Contact: Irritating to eyes.
Chronic Exposure: Repeated contact may cause allergic reactions in very susceptible persons.

6.3.5 Potential Health Effects of Potassium Dihydrogen Phosphate, Anhydrous, $KH_2PO_4$
Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion: Ingestion may cause gastrointestinal irritation and diarrhea.
Skin Contact: May cause skin irritation.
Eye Contact: May cause eye irritation.
Chronic Exposure: No information found.

6.3.6 Potential Health Effects of L-Ascorbic Acid, $CH_2OHCHOHCHCOH:COHCOO$
Inhalation: May cause respiratory tract irritation. Low hazard for usual industrial handling.
Ingestion: Ingestion of large amounts may cause gastrointestinal irritation. Low hazard for usual industrial handling. Large doses may cause diarrhea and acidification of the urine which may cause stones in the urinary tract.
Skin Contact: May cause skin irritation. Low hazard for usual industrial handling.
Eye Contact: May cause eye irritation.
Chronic Exposure: No information found.

## 7.0 Required documents
N/A

## 7.1 Input documents
Assay protocol.

## 7.2 Output documents
i. Absorbance data file in text format with timestamp of the read associated with the file.
ii. Saved data workup from Water Chemistry Template Macro.

## 8.0 Document control

## 8.1 Revision history
R0 – Initial Release – <Editor name> <Date>
R1 – <Editor name> <Date>

## 8.2 Document approval
<Name> <Approval date>

## 8.3 Document reviewers
<Name> <Last reviewed date>
<Name> <Last reviewed date>

## 9.0 Risk analysis
<Owner> <RPN>