

## **Procedure for Mineral Digestion**

### **Materials:**

Micro-kjeldahl flasks calibrated to 110 ml  
Spatula and brush  
Digestion apparatus  
Storage tubes (50 ml)

### **Reagents:**

**2 N Hydrochloric acid** (167 ml 12 N hydrochloric acid (EMD Cat #HX0603-3, FW 36.46, CAS # 7647-01-0) per liter 18 MOhm water)

### **Procedure:**

1. Use a spatula and brush to quantitatively transfer the ash (reserved from original dry matter sample) from the aluminum pan into a micro-kjeldahl flask.
2. Add 20 ml of 2 N HCl from preset repipette (be sure to wash down inside of flask with acid).
3. Place on digestion rack. Turn on the exhaust fan, the water aspirator, and the burners set to 3 or 4.
4. After solution comes to a boil, continue to boil for 10 minutes, swirling flask occasionally during this time (do not boil dry).
5. Unplug unit and let flasks cool slightly. Transfer to micro-kjeldahl rack.
6. Dilute with 18 MOhm water to 110 ml mark. Stopper and mix well by inversion. Pour into storage tubes and allow ash to settle overnight. This is the initial 110 dilution.

## Procedure for Mineral Determination (Calcium, Magnesium, Potassium)

### Materials:

Test tubes (20-30 ml)  
Pipettes (100-1,000 ul)  
Volumetric flask, 1,000 ml  
Atomic Absorption Spectrophotometer

### Reagents:

#### **Lanthanum oxide solution: 5% (w/v) in 25% HCl**

Wet 58.65 g lanthanum oxide ( $\text{La}_2\text{O}_3$ ) (Aldrich Cat # 199923-500G, FW 325.81, CAS # 1312-81-8) with 18 MOhm water. Slowly add 250 ml 12 N hydrochloric acid (37.7%) (J.T. Baker Cat #9530-33, FW 36.46, CAS # 7647-01-0) while swirling. Dilute to 1,000 ml with 18 MOhm water.

### Calcium Determination:

Various dilutions of the digested sample must be made to place the absorption of the elements in the working range of the instrument. The dilutions for calcium, magnesium, and potassium include lanthanum oxide to reduce chemical interference, which may occur with the presence of phosphorus in the sample.

1. Make dilutions using calibrated pipettes.
  - a. 2,750 dilution: 400  $\mu\text{l}$  of initial 110 + 2 ml  $\text{La}_2\text{O}_3$  + 7.60 ml 18 MOhm water in a 30 ml tube and vortex
  - b. 11,000 dilution: 100  $\mu\text{l}$  initial 110 + 2 ml  $\text{La}_2\text{O}_3$  + 7.90 ml 18 MOhm water in a 30 ml tube and vortex
2. **Use the atomic absorption spectrophotometer only with supervision.** Set the auto-zero with a blank solution containing 1%  $\text{La}_2\text{O}_3$  in 5% HCl. Set the standards of 2.00 ppm and 5.00 ppm using standards that contain  $\text{La}_2\text{O}_3$ .
3. Mix 2,750 dilution on vortex mixer. Read.
4. If reading is above 5.00, use an 11,000 dilution. If it is below 2.0, you have to make a 367 dilution.
  - a. 367 dilution: 3.00 ml initial 110 + 2 ml  $\text{La}_2\text{O}_3$  + 5.00 ml 18 MOhm water

### Calculations:

	A	B	C	D
File #	Conc. of Unk. Soln. (PPM)	Dilution	Dry Wt of Sample	% Ca
#100	4.54	2,750	1.8639	0.6698

$$\% \text{Ca} = \frac{\text{Conc (ppm)} \times \text{dilution} \times 0.0001}{\text{Dry Sample wt. (g)}}$$

$$D = \frac{A \times B \times 0.0001}{C}$$

**Reference:** AOAC method # 968.08, Association of Official Analytical chemists, 18<sup>h</sup> Ed., Revision 3, 2010.

## Determination of Phosphorus in Animal Feed

**Note:** This procedure is not applicable to feeds or mineral mixes containing monobasic calcium phosphate.

### Reagents:

#### **Phosphorus digestion solution:**

Dissolve 33.3 g Sodium molybdate (MCB Cat # SX690, FW 241.97, CAS # 7631-15-0 or Baker Cat # 3764-01, FW 241.95, CAS # 10102-40-6) in 1,000 ml 18 MOhm water; add 1,000 ml concentrated sulfuric acid (96%) (VWR Cat # 2876-46, FW 98.08, CAS # 7664-93-9).

#### **Ammonium vanadate solution:**

5.0 g Ammonium vanadate (Fisher Cat # A714-500, FW 116.98, CAS # 7803-55-6) dissolved in 1,500 ml hot 18 MOhm water. Add 40 ml concentrated nitric acid (70.7%) (VWR Cat # JT9598-34, FW 63.01, CAS # 7697-37-2). Dilute to 2,000 ml with 18 MOhm water in a volumetric flask.

#### **Ammonium molybdate solution:**

50 g Ammonium molybdate, 4-Hydrate (Baker Cat # A674-10, FW 1235.86, CAS #12054-85-2) dissolved in 1,000 ml 18 MOhm water in a volumetric flask.

#### **Phosphorus standard solutions:**

**Stock solution** – 2 mg P/ml – dissolve 8.788 g  $\text{KH}_2\text{PO}_4$  (Mallinckrodt Cat # 7100, FW 136.09, CAS #7778-77-0; MCB Cat # PX1565, FW136.09, CAS # 7778-77-0; or (anhydrous) Sigma Cat # P-5379, FW 136.1, CAS #7778-77-0), in 18 MOhm water and dilute to 1 liter.

**Working solution** – 0.1 mg P/ml – Dilute 50 ml stock solution to 1 liter with 18 MOhm water.

### Preparation of Standard Curve:

Transfer aliquots of working standard solution containing 0.3, 0.5, 0.8, 1.0, and 1.5 ml Phosphorus working solutions to 30 ml test tubes. Add solutions listed below, dilute to a volume of 10 ml with 18 MOhm water, and vortex. Let stand 10 minutes.

### Preparation of Samples:

Pipette 1 ml of digested and diluted samples containing 0.3 to 1.5 mg P into 30 ml test tubes.

Add: 0.5 ml phosphorus digestion solution  
1.0 ml ammonium vanadate solution  
2.0 ml ammonium molybdate solution

Dilute to 10 ml with 18 MOhm water and vortex. Let stand 10 minutes, and then read absorbance at 400 nm. The spectrophotometer must be zeroed with a blank before standards and samples are read. Analyze the standard curve using simple linear regression. Determine Phosphorus concentration using standard curve information.

**Reference:** AOAC method # 965.17, Association of Official Analytical chemists, 18<sup>h</sup> Ed., Revision 3, 2010.