

## Ammonium extraction procedure – SOP

*Concept:* Extracted KCl samples containing ammonium are kept frozen for long-term storage. Samples are thawed and run with an assay on a microplate reader to determine  $\text{NH}_4^+$  concentrations using a Citrate Reagent, a 2-Phenylphenol-Nitroprusside Reagent (PPS), and a Buffered Hypochlorite Reagent.

### **Primary safety concerns:**

*Acid bath:* This procedure involves working with dilute hydrochloric acid. The primary safety concern is exposure to bare skin or eyes.

*Precautions:* Wear protective eye wear, rubber gloves, lab coat, long pants, and closed-toe shoes when handling hydrochloric acid. When mixing solutions, always pour acid into water. Do not pour waste liquid down the sink. Emergency eye wash station is available outside the west laboratory entrance. Dispose of waste liquid in labeled 5-gallon plastic bins for EHS Hazardous Waste Pick-up.

### **Procedure:**

Nitrogen assays (in the lab):

1. In a **clear plate**, pipette 70  $\mu\text{L}$  of sample or standard into each well in triplicate.
2. Pipette triplicates of at least two (2) check standards into available wells as shown above. These check standards should be the two middle standards from the standard curve. If the standard curve is accurate, check standards should read close to their actual concentration under the “Calculated ppm-N  $\text{NH}_4^+$ ” column on the nutrient spreadsheet. Add check standards as if they were regular samples with respect to pipetting order. They should be the last two “samples” you pipet on the plate. Add all reagents to the check standard wells as if they were normal samples.
3. Add 50  $\mu\text{L}$  of citrate reagent to each well and allow it to react for approx 1 minute.
4. Add 50  $\mu\text{L}$  of PPS-nitroprusside reagent to each well.
5. Add 25  $\mu\text{L}$  buffered hypochlorite reagent to each well. Make sure any precipitate that formed upon adding citrate or PPS reagents dissolves completely. The buffered hypochlorite reagent must be added *after* the PPS reagent.
6. Add 50  $\mu\text{L}$  nanopure to each well.
7. Gently tap the corner of each plate to mix well. Plates may be stacked and stored making sure the top plate is covered. Incubate for 2 hours.
8. Turn on plate reader and allow it to warm up (1-2 minutes) before opening software.
9. Open Plate Reader software. Insert plate onto plate reader tray and open Ammonium Protocol. Fill in labels for “Plate Layout” if desired and read plate. Standard curve will be 1<sup>st</sup> order. Read plate at 660 nm.

Upon completion, samples are discarded in a sealable liquid waste container and disposed of by USU EHS Hazardous Waste Pick-up (435-797-2892).

**Emergency Contact:**

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