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Based on literature and intuition

HOLTGRIEVE ECOSYSTEM ECOLOGY LAB
PROTOCOL TO MIX AMINO ACID STANDARDS

INTRODUCTION

This document describes how to mix amino acid standards to the correct molarity to be used in the "Operate NACHO for Amino Acid Analysis" protocol.

SAFETY

You will be using 0.1N HCl in this protocol. Please read the MSDS for this chemical and for the target standards to be mixed prior to using and wear appropriate protective equipment including gloves, safety glasses and a lab coat.

MATERIALS

- 25mL volumetric flask
- Clean amber dram vials (same number as the number of standards you plan to mix)
- Scale (HEEL – FSH 232)
- Weigh paper
- Plastic or metal spatula (to transfer powder)
- d15NAA_Standards.xlsx spreadsheet
    - Stored on the HEEL drive within the "CSIA-N" folder under "Projects" (HEEL→Projects→CSIA-N)

REAGENTS

- 0.1N Hydrochloric Acid

MIXING STANDARDS

1. HEEL amino acid standards are mixed to a 0.05 molarity with the exception of tyrosine, which is mixed to a 0.0125 molarity to avoid dissolution. Download the d15NAA_Standards.xlsx spreadsheet and refer to it as you're mixing each standard.

2. Weigh out the mass detailed in the yellow "target mass (g)" column using a spatula to transfer the powder onto weigh paper. NOTE: be careful, the powder is REALLY light, so it is easy to add too much or too little powder. Enter the mass into the "actual mass

(g)" column. The molarity should be calculated under the "calculated molarity (M)" column.

3. Transfer the powder from the weigh paper into a 25mL volumetric flask (again be careful since the powder is light)and fill the flask to the white calibration line with 0.1N HCl. Cap and mix/invert the flask until all the powder is dissolved.

4. Transfer the mixed liquid standard to a clean amber dram vial and label with the name of the standard and the date it was mixed.

5. Repeat the process for all the target standards.

6. Follow the HEEL protocol to "Operate NACHO for Amino Acid Analysis" starting at step 2 under "preparing standard mixtures."

NOTE: With brand new standards (recently purchased) be sure to also run ten replicates for bulk isotope analysis and enter the nitrogen value under d15N (per mil vs air) in the spreadsheet. Also note that the target mass for EA in the spreadsheet is calculated for running at IsoLab. The target mass may vary on different machines so be sure to have the correct mass out for the target machine.