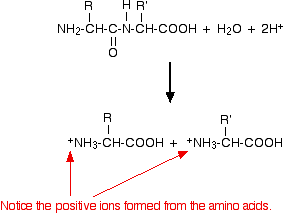
Acid Hydrolysis Procedure

**Purpose**: Acid hydrolysis cleaves the N-C peptide bonds in protein to free amino acids for subsequent analysis.

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**Start Up – First thing in the morning**

* Turn on Acid Hydrolysis Oven in CI324 using the power button on the front panelA picture containing indoor, oven, cabinet, wall

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* Load the Oven with aluminum blocks to hold sample vials and insert a thermometer that can read up to 150°C in the center block to monitor actual temp
* Set the oven to 110°C – Press the Menu button (temp icon will appear, circled above), Press Menu again (temp icon will blink), use arrows to change temp to desired set point, Press Menu again to initiate

**Preparing samples to acid hydrolysis:**

* Homogenize samples with a mortar and pestle to a fine powder.
* Weigh powdered samples into acid hydrolysis vials, record weights
* Add enough 6N HCl to vial to cover samples (minimum 0.5ml)
* Flush vials with atmosphere of N2 (3-4 seconds) and cap with black Teflon-lined caps quickly. Give every cap one extra check for tightness. The cap should gradually squeeze down to a stop.
  + - Note: if the cap feels like no resistance and then stops abruptly, it probably did not seal well. Use a glass pipette to transfer to a new 4ml vial and new cap.

**Acid hydrolysis: 110°C for 20 hours**

* Place vials in heating blocks inside acid hydrolysis oven at 110°C for 20 hours. After 5-10 mins, check that all caps are still tight and the liquid has not gone down at all (Caution: vials are hot!).
  + - Note: If the liquid level has decreased after check, place that vial in the freezer to cool, add enough acidified isopropanol to reach 1ml, and then transfer to a new vial and cap. Place back in the block for 20 hr. Again, check caps after 5-10 mins.

**Blow down reagent:**

* After 20 hrs, move all vials to the freezer to cool.
* Turn on reactivap to 60°C using the HEAT knob. Rinse however many reactivap needs you need (max 18 for both systems) using ethanol from the squirt bottle. Flush the needles with N2 prior to use to remove ethanol from inside the needles. Open the N2 cylinder valves (A, B) then open the corresponding valco valve (C). Close the valco valve again until ready to use.
* Label new 4ml vials with sample ID and AH (for acid hydrolyzed) for transferring samples after hydrolysis
* When vials are cool, use glass pipettes to transfer solution to new labeled 4ml vials and place vials in the reactivap to blown off reagent. Lower needles to ~1cm above liquid height. This typically takes ~30 mins. After 20 mins, check frequently. Remove vials that are dry and continue blowing down remaining vials until all are dry.
  + - Note: Make sure that N2 cylinder is open, only the reactivap you are using is open at the valvo valve, and there are plugs on any of the ports not in use.
* Once dry, remove vials from the reactivap, cap with new green Teflon-lined caps and store in freezer until ready to derivatize.

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**Clean up:**

* Turn acid hydrolysis oven down to 50°C (reverse of instructions above), turn off reactivap if not continuing on to derivatization immediately, and shut off N2 tanks (A, B, C sites)
* Make sure 6N HCl, ethanol, ethanol waste, and pipettes are all put away.