

CAU Laboratory Analysis SOP revised: September 2015

I. PREPARATION

1. Assign a unique ID to both plates of each Calcification Accretion Unit (CAU) using the **CAU Plate ID** naming convention *SiteID_RecoveryYear_4-digitSerial#_PlateLocation* ("U" - upper plate, "L" - lower plate)
e.g. **PAL01_2012_5319_U**
PAL01_2012_5319_L
2. Using a pencil, **pre-label the following with the CAU Plate ID (e.g. PAL01_2012_5319_U):**
 - a) Paper Tray A - blank index card (5x8) folded into 5x5 inch paper tray
 - b) Paper Tray B - same as above
 - c) Filterpaper 11cm diameter, #1, >11µm particle retention (e.g. Whatman No.1001-110)
3. Insert the corresponding Filter paper into the fold of Paper Tray B and store in a container. Place "Paper Tray A" in a separate container. Store both containers away from moisture and direct sunlight. See Step 8 below for pre-weighing instructions.
4. Print **Glass Dish Labels** on waterproof paper using the template: *CAUTemplate_DishIDLabel.xlsx*. Attach each label on one side of the glass dish with tape.
5. Print the **Plate Photo ID tags** on waterproof paper using the template: *CAUTemplate_PhotoIDTag.xlsx*
There are 4 tags, one for each surface of the CAU to be photographed:
Upper Plate, Top Side
Upper Plate, Bottom Side
Lower Plate, Top Side
Lower Plate, Bottom Side

An example of a plate photo ID: *CAU_PAL01_2012_5319_U_T*
6. Pre-label **re-sealable plastic bags** (1 quart) with the CAU Plate ID. The filter residue will be stored in this bag for archiving
7. Using the **lab data spreadsheet**, *CAUTemplate_LabDataSpreadsheet.xlsx*, enter the CAU Site IDs (e.g. PAL01) in Column A and the 4-digit CAU Serial #s (e.g. 5319) in Column B
8. Using an analytical balance, **pre-weigh** the following and record the value (up to 3 decimal points) in *CAUTemplate_LabDataSpreadsheet.xlsx*:
 - a) Paper Tray A - record the value in Column H ('Paper Tray A Weight [g]')
 - b) Paper Tray B + Filterpaper - record the value in Column I ('Filter & Paper Tray B Dry Weight [g]')
9. To calculate the "**Soak Time**" of each unit, record the CAU "Deployment Date" and "Recovery Date" in Column CA and CB, respectively. Record any other additional field data in the spreadsheet (e.g. coordinates, depth, comments, etc.)
10. Oven (Heratherm OGS-180 – <http://www.thermoscientific.com/content/tfs/en/product/heratherm-general-protocol-ovens.html>) settings:
Temperature: 60 °C (140F)
Damper: level 3 (mid-high)

11. Prepare 5% HCl acid solution in 2-liter bottles

One batch (20-30 units) of CAUs will require about 10-15, 2-liter bottles of 5% HCl acid solution in order to completely dissolve calcified materials. More may be needed depending on the amount of accreted CaCO₃.

To dilute full-strength HCl acid (1.18 g/mL, 36.5 molar mass, 37% conc.) into 5% solution, use the recommended ratio below:

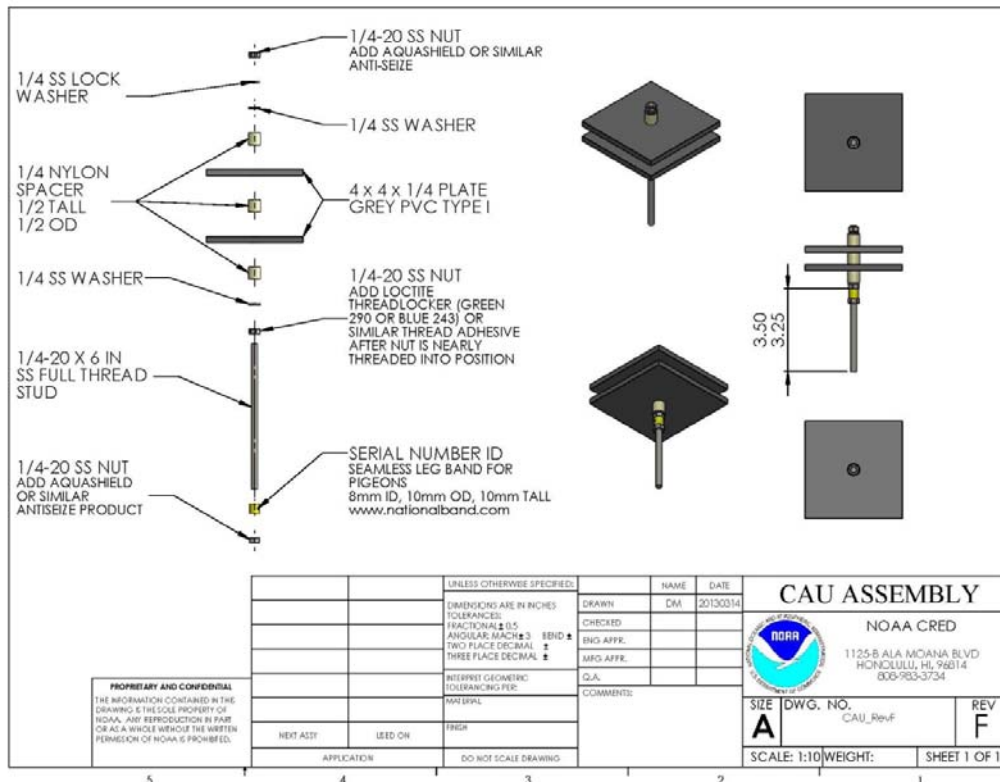
2L stock: 225 ml HCl
1775 ml deionized/distilled H₂O

1L stock: 112.5 ml HCl
887.5 ml deionized/distilled H₂O

* For more info, refer to CAUSOP_HCIDilutionPrep.docx

Server folder location: T:/general/ProjectGroup/Ocean Acidification/CAUs/Analysis/Analysis Overview Documents/Methods & SOP/Misc Processing SOP

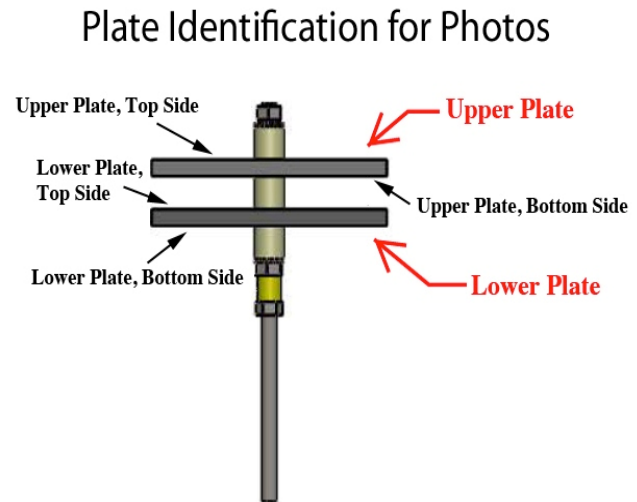
II. Calcification Accretion Unit (CAU) schematic



III. CAU Processing Steps (see Section IV for timeline)

A. Disassembling and photo-documentation

1. Thaw a batch of CAUs (typically 30-50 units) for at least 12 h. Do not thaw over water.
2. Disassemble the plates of each CAU by removing the hex nuts and washers using two wrenches (one to hold the unit steady). Make sure to maintain plate identity (upper vs lower plate, top vs bottom side of plate) and the CAU Plate ID.
3. Carefully rinse each plate in a saltwater rinse basin (if saltwater is not readily available, freshwater may be used) to remove loose sediment, sand and mobile fauna. Discard any mobile fauna that may have fallen off during rinsing.
4. Fill each pre-labeled glass dish with saltwater. Again, if saltwater is not readily available, freshwater may be used.
5. Place each CAU plate with the **top plate facing upwards** into its corresponding glass dish. Ensure that the plate and any encrusting fauna are completely submerged in water. Add more water if necessary.

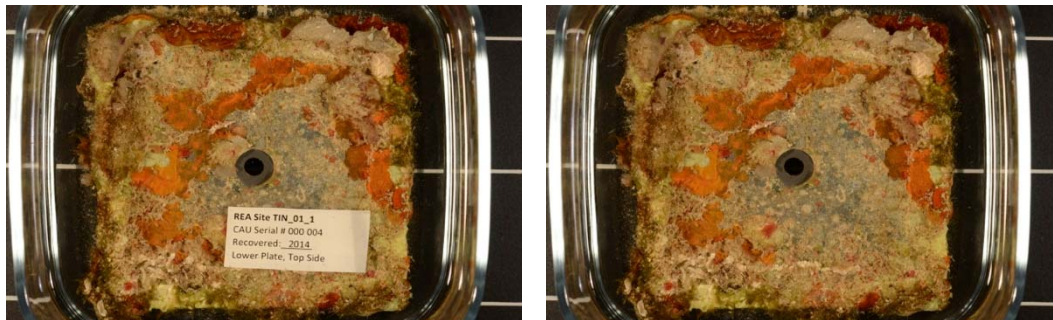


6. Attach a DSLR camera to a camera copy stand or a tripod. Arrange lighting such that the plate is fully illuminated and no glare is visible to the camera

**If using a Nikon D7000 camera mounted on a copy stand kit with attached lights, see CAUSOP_NikonD700Settings.docx to view the recommended settings (f/8, ISO400, AWB).*



7. It is recommended to tether the camera to a computer and save the photos directly to the computer's local drive (while using the camera's SD card as a backup drive). Designate a folder where the CAU photaset will be stored directly. It is recommended to group the CAU photaset folders within a Site folder (e.g. PAL01 \ PAL01_5319, PAL01_5320, PAL01_5321).
8. Photograph the top and bottom of each plate, first with the Plate Photo ID tag and then a 2nd image without the tag. (If pieces of calcified material fall off during the photographing process, retain the pieces with the plate to be used during the decalcification process).



9. From the designated CAU photoset folder, confirm that both plate photos (with and without the Plate Photo ID tag) are in focus. After photographing both sides of each plate, each CAU photoset folder should have 8 photos. The photos should be later on renamed with the following naming convention:

a) **With Plate Photo ID tag:**

CAU_SiteID_RecoveryYear_4-digitSerial#_PlateLocation ("U" - upper plate or "L" - lower plate)_PlateSurface ("T" - top plate or "B" - bottom plate)_ID.jpg

b) **Without Plate Photo ID tag:**

CAU_SiteID_RecoveryYear_4-digitSerial#_PlateLocation ("U" - upper plate, "L" - lower plate)_PlateSurface ("T" - top plate, "B" - bottom plate).jpg

Example:

CAU_PAL01_2012_5319_U_T_ID.jpg -- Upper Plate, Top Side with tag
CAU_PAL01_2012_5319_U_T.jpg -- Upper Plate, Top Side
CAU_PAL01_2012_5319_U_B_ID.jpg -- Upper Plate, Bottom Side with tag
CAU_PAL01_2012_5319_U_B.jpg -- Upper Plate, Bottom Side
CAU_PAL01_2012_5319_L_T_ID.jpg -- Lower Plate, Top Side with tag
CAU_PAL01_2012_5319_L_T.jpg -- Lower Plate, Top Side
CAU_PAL01_2012_5319_L_B_ID.jpg -- Lower Plate, Bottom Side with tag
CAU_PAL01_2012_5319_L_B.jpg -- Lower Plate, Bottom Side

10. After photographing all units, if the CAUs were submerged in seawater, carefully rinse each plate in freshwater. If the CAUs were submerged in freshwater, skip the rinsing process.

11. Be sure to remove all hex nuts and washers that may still be attached at the center of the plate. Hex nuts and washers can be covered by calcified material and difficult to see. Inspect each side of each plate to ensure that there are no washers or hex nuts present.

If a hex nut or washer is covered in calcified material, remove the hardware and chip off the calcified material from the hardware. Retain the removed material with the rest of the plate.

12. Place each plate and any dislodged pieces of calcified material on its corresponding Paper Tray A and on to a drying rack. The drying rack can be left in a fume hood to air dry at room temperature (minimum of 24 hours). The plates can continue to air dry at room temperature, indefinitely, until they are dried in the oven.

Drying at room temperature before placing in the oven can help reduce the time the plates remain in the oven.

Wash and rinse the glass dishes but keep the labels on. The same glass dishes will be used during the HCl decalcification process.

B. Determining the weight of the CAU plate

1. After a minimum air-drying period of 24 hours, place the **plate assembly (plate, dislodged pieces of calcified material and Paper Tray A)** in the oven to dry at 60°C for 24 hours.

Check that the internal temperature remains consistent. If not, minor adjustments should be made to the oven temperature and damper settings.

2. After 24 hours, remove the plate assembly and allow it to come to room temperature for 1 hour.
3. Weigh the **entire plate assembly**.
4. On the lab data spreadsheet, record the weight to at least 3 decimal places under Column M ('Drying 1 [24 h] [paper tray + CAU plate] [g]').
5. Return the plate assembly in the oven.
6. Dry the CAU plates for another 24 hours
7. Remove the plate assembly and allow it to come to room temperature for 1 hour.
8. Weigh the **entire plate assembly**.
9. On the lab data spreadsheet, record the 2nd weight to at least 3 decimal places under Column O ('Drying 2 [48 h] [paper tray + CAU plate] [g]')
10. Compare the current weight from the previous weight:
 - a) If the difference in weight is less than 0.1g, the plates are considered dry and no further drying needs to occur.

** On the lab data spreadsheet, conditional formatting has been applied to the columns labeled **Mass Lost (g)**. The cells turn green if the difference in weight is less than 0.1g*
 - b) If the difference in weight is greater than 0.1 g, return the plates to the oven for another 24 hours
11. Repeat III.B Steps 5-10 until the difference in weight is less than 0.1 g

C. Decalcification of CaCO₃ in Hydrochloric Acid

1. Ensure that the plate is fully dry (mass lost between last two weighing will have been less than 0.1g) and the weight of the plate assembly and Paper Tray A has been recorded.
2. Place the individual plates in the pre-labeled glass dishes. Retain Paper Tray A and place under the glass dish.
3. Chip off large pieces of calcified materials (e.g. CCA, coral, shells, etc) and grind using a mortar and pestle. Return the pulverized forms in the glass dish.
4. Fill the glass dish midway with 5% HCl solution and/ until the entire CAU plate is fully submerged in acid. Add HCl slowly as the solution may overflow when bubble formation/decalcification begins.
5. When decaclification has slowed (12-24 hours):



- a) Scrape leftover material from the CAU plates with a razor blade into the glass dish. (See III.C, Step 8-15)
 - b) Grind pieces of calcified materials that may still be present and return in the glass dish.
 - c) Using acid-resistant gloves, sort through the scraped fleshy material in the glass dish and feel for any calcified materials.
 - d) Some calcified material can be completely covered by a boundary layer of fleshy material. If present, manually tease the fleshy material and grind the calcified material to allow decalcification to continue.
 - e) If there are more calcified materials in the dish, but no obvious decalcification occurring (no bubble formation), decant the used acid solution and drain the fleshy material into another pre-labeled container (beaker, glass dish, etc). Add new 5% HCl solution in the first glass dish to continue dissolving the remaining calcified materials.
6. Repeat III.C, Steps 5b-e until all CaCO_3 is fully dissolved. Additional 5% HCl solution may need to be added 2-4x, depending on the amount of CaCO_3 present.
 7. Using 5% HCl in a squeeze bottle, thoroughly rinse the tools (gloves, mortar and pestle, razor/scraper) into the glass dish.
 8. When the CAU PVC plates have been scraped clean (fleshy/calcified material completely removed from either side and edges of the plate), rinse all sides of the plates with 5% HCl into the glass dish.
 9. Place the CAU PVC plate on its corresponding Paper Tray A. When dry, label the plate with the CAU Plate ID (e.g. PAL01_2012_5319_U) using a permanent marker.

Paper Tray A can be discarded after the CAU PVC plates have been labeled.

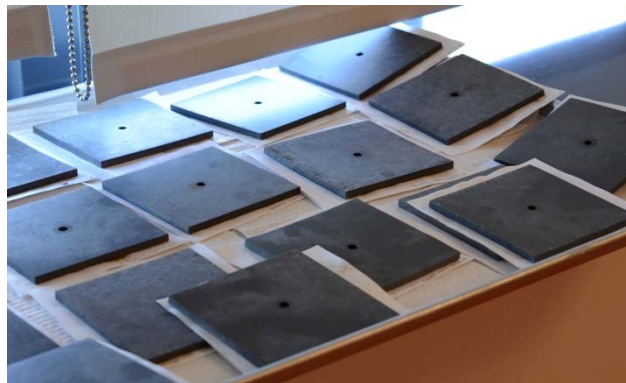
10. Scrub and rinse the CAU PVC plates in freshwater. Pat to dry.

11. Place the rinsed CAU PVC plates in the oven at 60°C for 24 hours.

12. Remove the plate from the oven and allow to come to room temperature for 1 hour.

13. Weigh the CAU PVC plate.

14. On the lab data spreadsheet, record the weight to at least 3 decimal places under Column K ('CAU PVC Dry Weight [g]').



15. Bind the CAU PVCs together using painter's/masking tape and put away for archiving.

D. Vacuum filtration of fleshy material

1. Using a 4-liter Buchner funnel, a plastic mesh layer (with attached pull string) and the corresponding pre-weighed filter paper, vacuum filter the acid bath and remaining fleshy material for each CAU plate. Use a freshwater squeeze bottle to rinse contents of the glass dish into the funnel.
2. If the volume of fleshy material is sufficient to clog the filter paper, a 2nd pre-labeled and pre-weighed filter paper (see III.D, Step 2, a-c) can be used to filter the remaining volume of acid bath and fleshy material:
 - a) Label an additional filterpaper and paper tray with its corresponding CAU Plate ID (e.g. *PAL01_2012_5319_U*)
 - b) In the lab data spreadsheet, record the weight of the 2nd filterpaper + paper tray to at least 3 decimal places in Column J ('Addtl Filter & Paper Tray B Dry Weight [g]')
3. If filtering has slowed down, use a spatula/teaspoon to gently scrape the surface of the filterpaper to allow more solution to filter through. Filtering can take >5 mins depending the amount of fleshy material in the acid bath.



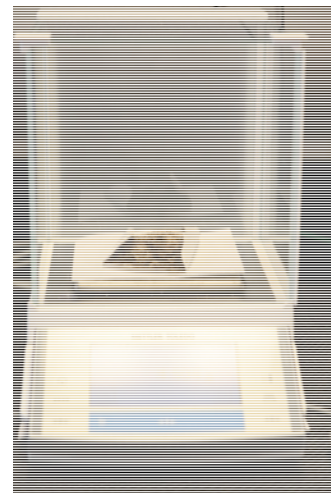
4. When filtration is completed, place the filterpaper on its corresponding Paper Tray B and on to a drying rack. The drying rack can be left in a fume hood to air dry at room temperature (minimum of 24 hours). The filterpaper can continue to air dry at room temperature, indefinitely, until they are dried in the oven.

E. Determining the weight of the fleshy material

1. After a minimum air-drying period of 24 hours, place the **filterpaper assembly (filterpaper and Paper Tray B)** in the oven to dry at 60°C for 24 hours.

Check that the internal temperature remains consistent. If not, minor adjustments should be made to the oven temperature and damper settings.

2. After 24 hours, remove the filterpaper assembly and allow it to come to room temperature for 1 hour.
3. Weigh the **entire filterpaper assembly**.
4. On the lab data spreadsheet, record the weight to at least 3 decimal places under Column AQ ('Drying 1 [24 h] [paper tray + filterpaper] [g]').
5. Return the filterpaper assembly in the oven.
6. Dry the filterpaper for another 24 hours
7. Remove the filterpaper assembly and allow it to come to room temperature for 1 hour.



8. Weigh the **entire filterpaper assembly**.
9. On the lab data spreadsheet, record the 2nd weight to at least 3 decimal places under Column AS ('Drying 1 [24 h] [paper tray + filterpaper] [g]').
10. Compare the current weight from the previous weight:
 - a) If the difference in weight is less than 0.1g, the plates are considered dry and no further drying/weighing needs to occur.

** On the lab data spreadsheet, conditional formatting has been applied to the columns labeled Weight of Fleshy Materials [g]. The cells turn green if the difference in weight is less than 0.1g*
 - b) If the difference in weight is greater than 0.1 g, return the plates to the oven for another 24 hours
11. Repeat III.E Steps 5-10 until the difference in weight is less than 0.1 g
12. After recording the final filterpaper assembly dry weight, place the filterpaper and Paper Tray B in its corresponding re-sealable bag for archiving.



IV. Processing Timeline

Plan A. 120 CAU units/~9 weeks

	Sun	M	T	W	Th	F	Sat	
Batch 1					Def	Proc	AD	Week 1
	AD	OD	W/O	W/O	W/O	W/Decal	Decal	Week 2
Batch 2					Def	Proc	AD	
	Decal	Sc/Decal	Sc/Decal	Filt	Filt	Filt	AD	Week 3
	AD	OD	W/O	W/O	W/O	W/Decal	Decal	
	AD	W/O	W/O	W/O	W/O			Week 4
	Decal	Sc/Decal	Sc/Decal	Filt	Filt	Filt	AD	
	AD	W/O	W/O	W/O	W/O			Week 5
Batch 3					Def	Proc	AD	
	AD	OD	W/O	W/O	W/O	W/Decal	Decal	Week 6
Batch 4					Def	Proc	AD	
	Decal	Sc/Decal	Sc/Decal	Filt	Filt	Filt	AD	Week 7
	AD	OD	W/O	W/O	W/O	W/Decal	Decal	
	AD	W/O	W/O	W/O	W/O			Week 8
	Decal	Sc/Decal	Sc/Decal	Filt	Filt	Filt	AD	
	AD	W/O	W/O	W/O	W/O			Week 9

		<i>Man Hours</i>
Def	<i>Defrost; prep lab</i>	2
Proc	<i>Process: Dismantle and photograph</i>	7
AD	<i>Air-dry</i>	
OD	<i>Oven-dry</i>	
Decal	<i>Decalcify (sit in 5% HCl bath)</i>	4
Sc/Decal	<i>Scrape and decalcify</i>	7
Filt	<i>Vacuum filtration process</i>	7
W/O	<i>Weigh (and oven-dry)</i>	1
W/Decal	<i>Weigh and decalcify (pour HCl; pulverize large pieces)</i>	7
	Total	35

Plan B. 120 CAU units/~13 weeks

	Sun	M	T	W	Th	F	Sat	
Batch 1					Def	Proc	AD	Week 1
	AD	OD	W/O	W/O	W/O	W/Decal	Decal	Week 2
	Decal	Sc/Decal	Sc/Decal	Filt	Filt	Filt	AD	Week 3
	AD	W/O	W/O	W/O	W/O	W/O		Week 4
Batch 2					Def	Proc	AD	
	AD	OD	W/O	W/O	W/O	W/Decal	Decal	Week 5
	Decal	Sc/Decal	Sc/Decal	Filt	Filt	Filt	AD	Week 6
	AD	W/O	W/O	W/O	W/O	W/O		Week 7
Batch 3					Def	Proc	AD	
	AD	OD	W/O	W/O	W/O	W/Decal	Decal	Week 8
	Decal	Sc/Decal	Sc/Decal	Filt	Filt	Filt	AD	Week 9
	AD	W/O	W/O	W/O	W/O	W/O		Week 10
Batch 4					Def	Proc	AD	
	AD	OD	W/O	W/O	W/O	W/Decal	Decal	Week 11
	Decal	Sc/Decal	Sc/Decal	Filt	Filt	Filt	AD	Week 12
	AD	W/O	W/O	W/O	W/O	W/O		Week 13

V. CAU Processing Equipment & Supplies list

Equipment/Supply	Brand and Model	Specs
Drying Oven	Heratherm Advanced Protocol Oven (OGH180, 220-240v, 60Hz) or Heratherm General Protocol Oven (OGS 180, 120v, 60Hz)	Gravity convection; 176 L, 6.2 cu ft; Interior (WxHxD) = 17.2 x 26.8 x 23.2 in Exterior (WxHxD)=25.2 x 36.2 x 29.1 Shelves: 2 supplied, 19 max
Digital SLR Camera	Nikon D7000	
60mm micro lens	Nikon AF-S Micro NIKKOR 60mm f/2.8 G ED	
Fume hood		benchtop, ducted laboratory hood
Wire Mesh Shelves	Heratherm Wire Mesh Shelf (Part No.50127763)	Includes 2 shelf support Max carrying capacity/shelf: 55 lbs
Copy Stand	Bencher Copy Mate II Halogen Copystand 900-30SC	Overall Dimensions: 20"W x 19"D x 3-1/2"H Includes two (2) adjustable halogen copylights and two (2) fixed copylight arms: Light Output: 300W each, 600W total Bulb: Ushio #1000896 - JCD120V-300WC
Analytical Balance	Mettler-Toledo XA Precision Balance (XA503S)	
Vacuum Filtration Pump	Vacuubrand Oil-free Diaphragm Pump (ME1) 50/60 Hz	Max pumping speed 0.4/0.5 cfm
Lab-grade rubber tubing	Tygon E-3603 (ACF00029)	Interior Diameter = 3/8" Outer Diameter = 5/8" Wall Thickness 1/8 "
Filter flask	Pyrex	4000 ml
Buchner funnel	Buchner Funnel 110 mm, glazed porcelain (Avogrado's Lab Supply, Item # 33065)	Glazed porcelain Inside Diameter = 110 mm Height = 172 mm Bowl depth = 44 mm Volume = 456 ml
Rubber filter aid	Rubber filter aid (Avogrado's Lab Supply, Item # FLTR01)	Inside Diameter = 32mm/1.2 inch Outside Diameter = 76 mm/ 3 inches
Squeeze bottles		
Tubing connectors	Tapered Tubing Connector 3/16 to 5/16" (Avogrado's Lab Supply, Item # KT511)	Unequal 3/16 to 5/16 and 1/4 to 1/2
Buchner funnel mesh discs	Buchner Funnel Discs 90 mm 5/pk (Avogrado's Lab Supply, Item # 242845-90)	Diameter = 90 mm / 3.54 inch Mesh size = 900 x 700 um * for use with 110 mm Buchner funnel
Filter paper	Whatman Qualitative Grade 1 Filterpaper Mfr #1001-110	11 cm diameter Pore size: 11 um
Razor scraper	Titan 12033	
Razor blade refills	Garvey (40475)	.02 inch thickness 5 x 2 x 1 inches
Glass dishes	Bormioli Rocco Frigoverre (25 ½ oz) Model # 387870MB2321990	6 x 6 x 2.5 inches Capacity = 25 ½ ounces
Resealable plastic bags	Ziploc Freezer Bags - Quart size	7" x 7 ¾"; Clear; 1.75 mil
Index cards	Pendaflex Oxford Index Cards, White, Blank 5x8	5 x 8 x 0.8 inches, white, acid free
Hydrochloric Acid	EMD Millipore Hydrochloric Acid (No. HX0603-75); 2.5L	Molar Mass: 36.46; Assay (HCl) approx. 36-38%

Glass bottles for 5% HCl storage	EMD Millipore Storage Jugs; 2.5 L	2.5 liter; narrow mouth with polypropylene caps
PPE		Lab Coat Chemical-resistant Disposable gloves Oven mitt Safety Glasses Closed-toe shoes