Protocol for Acid-Base-Acid Pre-treatment of Coal, Wood, and Organic Macrofossils
in preparation for graphitization

1. **Purpose:**
   The intent of this protocol is to provide instruction on how to prepare coal, charcoal, wood, and macrofossil samples for graphitization by cleansing the sample of any surface contamination of recent atmospheric carbon.

2. **Application:**
   Coal and TIRI wood are used as secondary standards for quality assurance in the radiocarbon laboratory. This method is used both for the pre-treatment of the designated secondary standards and preparing incoming samples of macrofossils, wood, or charcoal. This procedure is also the preliminary step to cellulose extraction of wood samples.

3. **Summary of Method:**
   The sample is subjected to hydration with 1N HCl, where carbonates are removed, followed by removal of humic acids with several 1N NaOH washes, removal of carbon gases with a second 1N HCl wash and a final rinse with nanopure water to a neutral pH.

4. **Interferences:**
   As in any radiocarbon lab contamination from ^{14}C poses a constant concern. Prepare the work area with a fresh layer of foil. Do not accept samples from labs that have not passed a swipe test.

5. **Apparatus/Equipment:**
   - **5.1** block heater (VWR 12621-104)
   - **5.2** 20-hole block module (VWR 13259-130)
   - **5.3** thermometer
   - **5.4** vortex
   - **5.5** 13 x 100 mm borosilicate tubes
   - **5.6** glass stir rod
   - **5.7** drying oven
   - **5.8** 3 mL transfer pipette (Samco Scientific 225)
   - **5.9** aluminum foil
   - **5.10** 2 150 mL beakers
   - **5.11** beaker for waste
   - **5.12** secondary containment for reagents
   - **5.13** timer
   - **5.14** TIRI wood sample
   - **5.15** coal standard
   - **5.16** fine-tipped forceps
   - **5.17** scalpel

6. Reagents:

6.1 1N HCl (Fisher Scientific SA48-1, or other equivalent. CAS# 7647-01-0).

6.2 1N NaOH (BDH 3222-1, or other equivalent. CAS# 1310-73-2).

6.3 nanopure water (18.2 M-Ohm)

6.4 ethanol

7. Safety Precautions:

7.1 Familiarize yourself with the MSDS for HCl and NaOH and be prepared should any spills occur.

7.2 Nitrile gloves, safety goggles (or other appropriate eye protection), close-toed shoes, and a lab coat must be worn while performing this procedure. Although the concentrations of hydrochloric acid and sodium hydroxide are low, direct contact with these chemicals can still cause skin irritation, severely burn the eyes, and damage clothing. The reagents are brought to a near-boiling temperature causing fumes to escape, and possible burns may result from the high temperature should the sample splash out or spill from the tube.

7.3 Neutralize the waste generated by adding sufficient sodium bicarbonate to the waste beaker. It can then be sewered with copious amounts of water. This step mitigates potentially harmful personal contact.

8. Procedure:

8.1 Coal standard and TIRI wood should be kept stored in a Secador, or low humidity containment. Incoming samples should be oven dried at 50^{o}C for 24 hours.

8.2 Have prepared sufficient borosilicate disposable culture tubes, size 13 x 100 mm. Prepared tubes have been baked in the muffle furnace at 400^{o}C for 2 hours. Keep tubes wrapped in foil and stored in a humid Secador (i.e. containing a tray of 1N NaOH to absorb nitrates) until ready for use.

8.3 Wipe clean the weighing area and place a fresh layer of aluminum foil on working surface.

8.4 Label a borosilicate tube with sample number and tare it.

8.5 Weighing out of the sample.

For the coal: Using fine-tipped forceps to pick out a granule of coal, weigh out approximately 2 mg into the sample tube. Do not use a powdery coal standard -- granules weighing 2-3 mg are ideal.

For the TIRI wood: use an ethanol-cleaned sharp scalpel and shave off 4 - 5 mg of wood fiber from the wood fragment and weigh the shavings into a borosilicate tube. To prevent contamination from skin oils do not touch the wood with your bare fingers. Wear nitrile gloves. The wood can also be wrapped in a piece of foil, with the portion of wood to be shaved exposed. Although this number will vary, expect that ABA-treated wood will have lost about 25% of its weight. At this time a precise sample weight is not necessary to record.

For wood samples that will be bleached following this procedure: same as for TIRI wood however shave off 20-40 mg of wood, from a single tree ring if possible. The larger quantity of wood is needed as there will be significant losses during the bleaching.

8.6 Begin ABA pre-treatment as follows:

8.6.1 Set heating block temperature to 95^{o}C.

8.6.2 Set all labeled sample tubes containing their respective samples in the block heater.

8.6.3 Using a 3 mL transfer pipette add approximately 3 mL 1N HCl to each tube. It is important that the acid come in complete contact with the sample.

8.6.4 Set timer for 20 minutes. During this time unwanted carbonates and some fulvic acids are removed. **Note:** *After a few moments the HCl begins to react with the sample as evidenced by small air bubbles that will form on the coal and wood surfaces. These can be removed by gently vortexing the sample tubes. This should be done with great care, as too vigorous a spinning with the vortex will cause the heating HCl to spin out of the tube.*

8.6.5 Aspirate off the HCl with a transfer pipette and discard aspirant in a beaker designated for liquid waste. ***Note:*** *It is important not to cross contaminate any of the samples. Each sample will have its own disposable transfer pipette for aspiration.*

8.6.6 To each sample add 3 mL of 1 N NaOH.

8.6.7 Set timer for 25 minutes. The NaOH washes will reduce the sample size somewhat, as will be evidenced by the solution turning to a dark yellow color. With each subsequent NaOH wash the solution becomes clearer as base-soluble humic acids are separated out.

8.6.8 Aspirate off the NaOH with a transfer pipette and discard aspirant in a beaker designated for liquid waste. ***Note:*** *It is important not to cross contaminate any of the samples. Each sample will have its own disposable transfer pipette for aspiration.*

8.6.9 Repeat steps 8.6.6 through 8.6.8 until supernatant remains clear.

8.6.10 Make note on the 'sample treatment card' for the sample the number of NaOH washes performed.

8.6.11 Using a 3 mL transfer pipette add 3 mL 1N HCl to each tube. Because the NaOH is hygroscopic and readily absorbs CO_2 from the atmosphere, the sample is again washed one time with 3 mL of 1N to remove any carbon gases that may have formed during the NaOH washes.

8.6.12 Set timer for 20 minutes

8.6.13 Aspirate off the HCl, transferring waste to the beaker.

8.6.14 Turn off the heating block.

8.6.15 Rinse each sample with 3 mL of nanopure water (ASTM Type I, 18.2 MΩ).

8.6.16 Set timer for 2 minutes

8.6.17 Aspirate off the water.

8.6.18 Repeat steps 8.6.15 through 8.6.17 two times or until the water pH is neutral.

8.6.19 Set heating block temperature to 85°C .

8.6.20 Lightly cover all samples with a foil tent so moisture can escape and allow samples to dry in the heating block overnight.

The following chart outlines the ABA steps:

Treatment	Solution	Time (min)	Temp (°C)
Acid	1N HCl	20	95
Base	1N NaOH	25	95
Base	1N NaOH	25	95
Base	Repeat base treatment until supernatant is clear		
Acid	1N HCl	20	95

Rinse	nanopure H_2O	1-2	Room temp
Rinse	nanopure H_2O	1-2	Room temp
Dry	None	Until dry	85

The pre-treated samples are now ready for weighing.

Following is an image depicting the set-up for the ABA procedure:

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