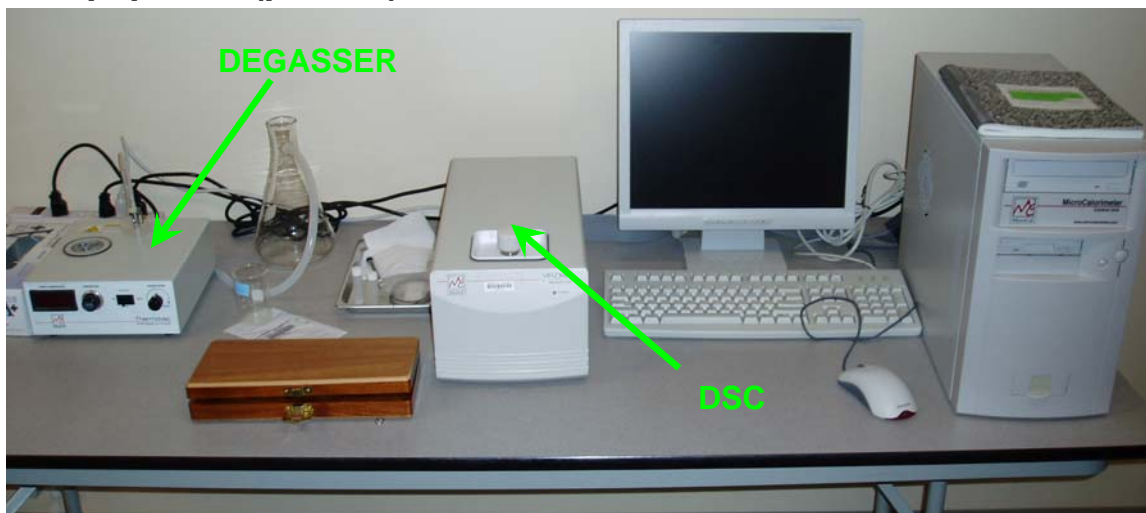


Protocol for Use of Differential Scanning Calorimeter (NSF/EPSCoR Proteomics Facility @ Brown University)

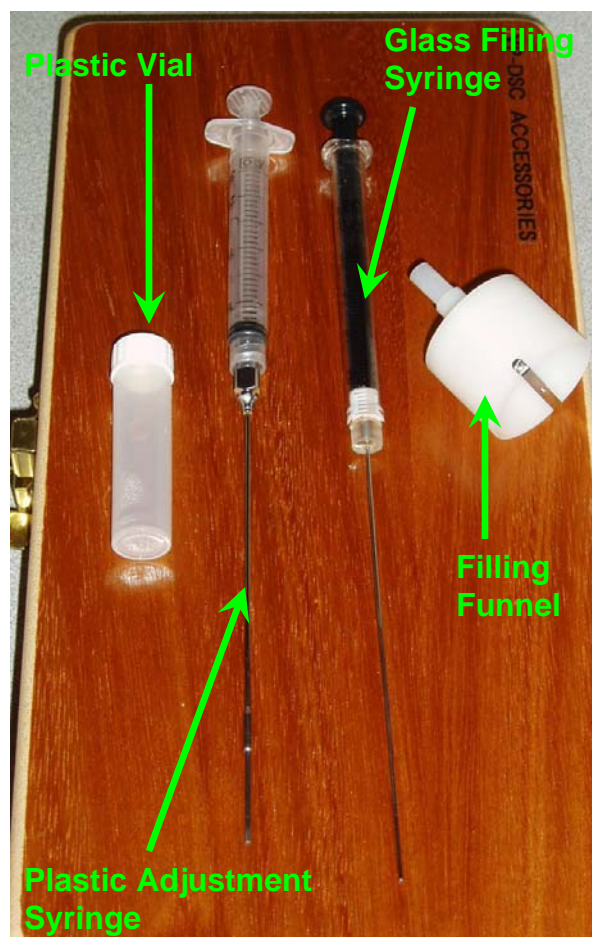
1. Equipment (photos)



2. Getting Started

Degasser

- Turn on the degasser.
- Make sure the metal valve on the top of the lid is closed.
- Set the temperature to 20-25 °C.
- Place a small stir bar in a couple of plastic vials.
- Fill vials 2/3 full with Milli-Q water.
- Place vials into slots on top of degasser.
- Turn the stirrer speed knob to 10 or 11 o'clock.
- Place the lid on firmly, and while pressing down turn on the degasser (**Note:** If you flip the switch to on, the degasser will run until switched off. If the switch is flipped to timer, the degasser will run for eight minutes).
- Degas the sample for 8-15 minutes.



- Turn off the vacuum.
- Open the metal valve on top of the lid in order to release the vacuum created underneath.
- Remove the plastic vials.

DSC

- Unscrew top.
- Remove contents of cells using the glass filling syringe. Slowly put the syringe into each one of the cells until it gently touches the bottom, then remove the liquid. Repeat. (**Note:** Be careful when putting anything into the cells. The machine is extremely sensitive.)
- Fill glass syringe with degassed Milli-Q water.
- Rinse out both cells with the Milli-Q water (3x).
- Remove contents of both cells using the glass filling syringe.

3. Buffer-Buffer Equilibration

Filling DSC

- Degas at least 2/3 of a plastic vial full of buffer the same way as described in the above section (**Note:** Buffer is what you are going to dilute your protein sample into, most likely what your protein was purified in).
- Fill the glass filling with syringe with ~0.7 mL of degassed buffer.
- Place the filling funnel firmly into one of the cells.
- Insert the syringe into the small whole in the bar of the filling syringe, and then into the cell itself (this places the tip of the syringe ~2 mm above the bottom of the cell).
- Very slowly, press down on the plunger of the syringe.
- When there is approximately half the buffer left in the syringe, add it to the cell with quick thrusts of the plunger (this is to dislodge any bubbles that may be caught at the bottom of the cell).
- Remove the filling funnel.
- Take the plastic adjustment syringe and gently put it as deep into the cell as it will go.
- Pull up on the plunger and remove as much liquid as it will allow.
- Follow the same steps in filling the other cell.
- Screw on the cap until it is hand tight.

Computer

- Turn on computer.
- Click OK when it asks for a password.
- Double click VP Viewer 2000 DSC shortcut on the desktop.
- Click OK when a prompt opens about the program Origin.
- Now VP Viewer and Origin are both open. VP Viewer is used to change the parameters and Origin is used to graphically model the data.

Parameters

In VP Viewer

Experimental Parameters

- Number of Scans → 20
- Post cycle thermostat → 10 °C

Scan Parameters

- Starting temp → 10 °C
- Final temp → 90 °C
- Scan rate → 90 °C/Hr
- Prescan thermostat → 15 mins
- Postscan thermostat → 0 mins
- Choose a file name, but leave the number and the .dsc.
- Click Start at the top of the VP program.

Running buffer-buffer equilibration

- After pressing the start button it takes a while for the program to get going. The temperature needs to drop down to 10 °C, and then there is a period of equilibration. Be patient. If scan hasn't started in half an hour, something is amiss.
- Once the scans have officially started (Origin will read at the top 'Scanning up to 90 °C), let it run over night. This allows the system to equilibrate.
- When you come back the next day, click on the button 'completed scans' in the Origin program.
- If all the scans look rather uniform (decent overlap) then you are ready to test your sample.
- Stop run in VP Viewer program if it hasn't stopped yet.

4. Testing a sample

Preparing sample

- Make ~4 mL of sample.
- You should use the buffer you used in section three to dilute the sample.
- Try and get the concentration as close to 0.1 mg/mL as possible.
- Degas the sample as outlined in section two (degas very well, 15-20 mins).
- Remove the buffer from the sample well (the sample well should be the one on the left, but there is an S and an R on the white base that surrounds the wells).
- Fill the sample cell with your sample, following the steps outlined in section 3.

Parameters

- Keep all parameters the same as in section three except change:
- Number of scans → 1.
- Change the file name.
- Press start.
- Once the scan is complete **SAVE IT** using Origin (they are very hard to find again if you forget to do this).

5. Clean-up

- Empty the cells
- Rinse using degassed Milli-Q water 3x.
- Clean the cell using 5% detergent solution, using the special equipment provided by Microcal.
- Fill cells with degassed Milli-Q water according to section 3.
- Return cap.
- Turn off DSC, computer, and degasser.