Simplified TA nanoDSC III protocol

STOP This protocol is for qualified users operating nanoDSC III in IBC 402 only. Dr. Jao accepts no responsibility for actions taken as a result of using this protocol. Reading the manufacturer's User's Manual is highly recommended.

Sample preparation:

1. For each cell, prepare >650 μL (700 μL is easier for loading) of solution, degassed at starting temperature for at least 10 min. under a vacuum of 500 mm-Hg.
2. Protein concentrations are usually 0.2 mg/mL to 1mg/mL.
3. Prepare 30-50 mL of buffer for baseline scans and cleaning purpose.
4. Please consult manager if you intend to use non-aqueous solutions.

Start up:

5. Power up the instrument if the instrument is not on.
6. Boot up the control PC and log on to Windows.

7. Make sure the internet is not connected then double click "Measurement and Automation" icon to establish connection between control PC and the instrument.
8. Initialize DSC control software by double-clicking "Launch DSCRun". The software contains three tabs: **Experiment Method, Monitor,** and **Data.**
9. Should the program fail to start, close the window and try again.
10. Basic parameters for a DSC experiment may be set up by loading a previously saved experiment method (.dsccfg).

Sample loading and start:

11. Drain water from reference cell, rinse the cell twice with your buffer and drain residual buffer.
12. Load buffer (degassed) to the cell carefully by a one-mL pipette:
    a. Attach one end of the capillary cell with a pipette tip.
    b. Withdraw 650 μL of degassed buffer and attach the pipette to the other end.
    c. Slowly dispense buffer to the capillary cell until the solution emerges from the outlet.
    d. Gently oscillate 3 times to remove bubbles.
    e. Slowly withdraw excess buffer so that only 5mm in heights of solution can be seen at the other outlet.

f. Detach the pipette (with pipette tip) without disturbing the solution.
g. Detach the other pipette tip slowly.
h. Fill black vinyl cap with buffer and cap one end of the capillary cell.
i. Fill sample capillary with same procedures described above a. to h.

13. Tighten the pressure handle.
14. Within the DSCRun control software, monitor the heat change on the **Monitor** page. Alter the plotting scale to be -15 to 20 μW and wait for the baseline to stabilize within 1 μW.
15. While on the **Monitor** page, pressurize the cell to 3 atm by clicking the pressurize button (Up arrow). Watch closely on the change of the heat baseline. Sudden change of the baseline >10 μW indicates bubbles in the cells and the cells need to be refilled.
16. Set up experiment parameters and start a set of 10 scans for conditioning the cells overnight. Please note that a temperature scan of **5-110** ℃ is usually sufficient for most protein samples. Never freeze your sample in filled cells. An equilibration period of 900 seconds is recommended for each scan.
17. After an overnight experiment of buffer/buffer scans, check for baseline repeatability. If the baseline is stable, stop the current conditioning experiment and wait for the temperature to come to idle temperature before depressurizing the cells.
18. Refill both cells with degassed buffer and start a blank experiment for one scan using the same parameters as for the conditioning experiment but a different filename.
19. While the cell temperature is coming down to the idle temperature, refill reference cell with buffer (optional) and replace sample cell with your sample solution (degassed) by following instructions of loading procedures 12 a.~12 h, except for the black cap for sample capillary which should be filled with sample solution before capping.
20. Tighten the pressure handle.
21. Start one scan using the same parameters as for the blank scan and provide the data a different filename.
22. Once the temperature comes down to idle, depressurize cells and proceed to the cleaning procedure.

Cleaning Procedure:

The following is adopted from Nano DSC Getting Started Guide.

23. Withdraw your sample from sample cell using 1-mL pipette and check for precipitates. Rinse sample cell with 1mL buffer three times with pipette.
24. Empty both cells and refill cells with 15% Decon 90 detergent solution. Cover the chamber with a damp paper towel and perform one scan cycle from 50 to 80℃ at 1℃/min. It is NOT necessary to apply pressure on the system.
25. When the temperature comes down to idle, close the DSCRun control program.
26. Empty detergent solution from both cells and pass 1L ddH2O through cells using flushing equipment and TA's degassing station as shown below.
27. When done, activate DSCRun control software.
28. Repeat step 24 to 26 except replacing detergent solution with 50% Formic acid. Please note that you should cover the chamber with a damp paper towel and perform one scan cycle from 50 to 80℃ at 1℃/min. It is not necessary to apply pressure on the system.
29. After passing 2L ddH2O through both cells, thoroughly clean all area that might have been contaminated by cleaning solutions.
29. Activate DSCRun control software and fill both cells with degassed ddH2O.
30. Perform 10 water/water scans from 10 to 110 ℃ at 1℃/min to check for baseline stability.
31. When the cells are clean, depressurize cells and leave the instrument on.
32. Turn off control computer and monitor.
33. For analysis, please refer to the **NanoAnalyze Software Guide.**