Simplified MicroCal iTC$_{200}$ protocol

STOP This protocol is for qualified users operating iTC$_{200}$ 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. Prepare 300 to 350 μL of the sample for the cell and 60 μL titrant for the titration syringe. It is advised to have at least 110 μL titrant to be able to perform an additional control experiment. The buffers in both the sample cell and the titration syringe must be **identical** to avoid heat of dilution. No degassing is necessary for iTC200 unless you are running at a very different temperature from room temperature.
2. Prepare 15 mL of buffer and 100 mL ddH_2O for cleaning purpose. Buffer should be filtered through a 0.22 μm filter before use.
3. If you don't know the binding constant of your system, use 1/5 of highest concentration of your macromolecules in cell for a start. 10μM~100μM is a good guessing concentration or use the **Experimental Design** tab of the iTC200 software to estimate according to your sample. Otherwise, you may calculate c value by the following equation: $c = n * M_{TOT} * 1/K_D$
   The optimum range for c is 10 <= c <= 100. Please refer to User's Manual Section 2.3 for details. The concentration of titrant in syringe should be at least 10-15 times higher if the stoichiometry is 1 to 1.
4. Consult manager if you intend to use non-aqueous solutions.

Start up:

5. Boot up the control PC.
6. Log in to Windows.
7. Power up the instrument.
8. Inspect syringe to check for possible crack near filling port.
9. After several seconds, initialize the iTC200 software by double click 

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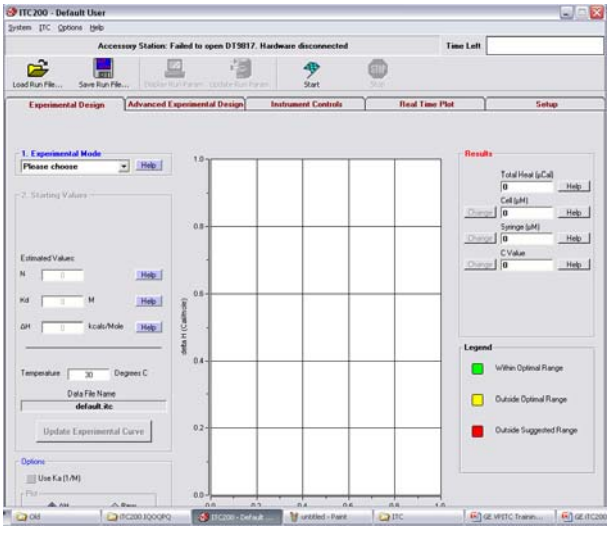
10. The software contains five tabs: **Experimental Design, Advanced Experimental Design, Instrument Control, Real Time Plot** and **Setup.** The

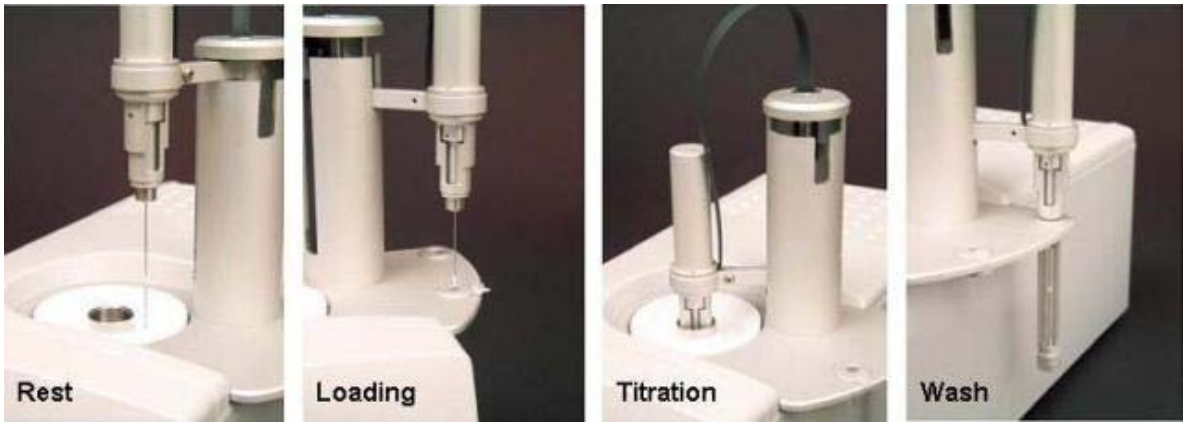


description of the tabs can be found in User's Manual Section 2.

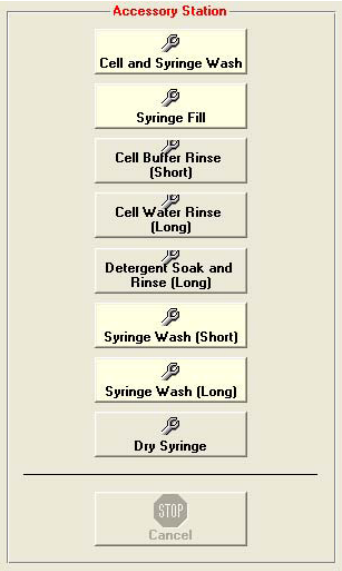
11. The parameters for an iTC run may be set up by calculations in the **Experimental Design** page or by manually input in the **Advanced Experimental Design** page.

Sample loading and start:

12. Set jacket temperature by thermostat control in the **Instrument Controls** tab.
13. To load sample to the sample cell, pull 300-350 μL of sample into the Hamilton syringe. Insert the syringe to the bottom of the sample cell and raise the tip about 1mm. Fill the sample cell slowly until the sample spills out the top of cell stem. Gently oscillate 3 times and stir to remove bubbles. Oscillate again if necessary. Drain excess sample.
14. Usually, the reference cell is filled with ddH_2O. If you'd like to fill the reference by yourself, please follow the same procedure as for the sample cell.
15. To load the titration syringe, place a PCR tube with at least 60 μL of titrant in the tube holder. Move the injector pipette to the "**rest**" position (figure below). Connect the syringe filling adaptor to the syringe **carefully**. In the **Instrument Controls** tab, click **Syringe Fill** and follow the pop-up instructions to fill the syringe using Washing Module. If the titrant does not fill properly to the top of the syringe, add 5 μL more in the PCR tube and perform **Syringe Fill** again at the loading position. This usually will remove the air bubble.
16. After loading the syringe, ***disconnect*** the filling adaptor and lower down the syringe in to the sample cell slowly.
17. Check all the parameters and titration table in the **Advanced Experimental Design**, including the data file name.



18. To start the titration experiment, click Start.
19. Watch for the baseline position, which should be close to the set reference power value and the nosie is around 0.005 μcal/sec.
20. You should rinse Hamilton syringe with buffer and water now.



Cleaning Procedure:

21. Place an empty PCR tube in the loading holder.
22. Move the injector pipette to the "**Load**" position and connect the syringe filling adaptor.
23. Click **Syringe Wash (long)** and *manually* rinse injection syringe at least 3 mL of your ***buffer***.
24. Rinse syringe with **10mL of water** till the syringe is clean.
25. Wet the syringe with 200 μL of **ethanol**.
26. Air blow dry the exterior of the syringe for 30 seconds and let the syringe drying process continue for at least 3 minutes at the "**Wash**" position**.**
    Important Note: **Dry Syringe** may only be used for drying, NOT for washing! Use **Syringe Wash (Long)** for washing**.** Please check the waste vial constantly during wash step.
27. Fill water bottle (or vial, the second one from left) with ddH_2O.
28. Withdraw your sample from sample cell using Hamilton syringe and ***manually*** rinse the sample cell at least 3 times by filling the cell with 350μL of your *buffer* oscillating up and down. Rinse the cell with 3 times of 350μL *water*.
29. Fix the syringe filling adaptor to the holder on the back of the washing module. Insert the **cell cleaning apparatus** into the sample cell and perform **Cell Water Rinse (Long)** at least twice In the **Instrument Controls** tab. Watch the waste vial carefully for waste level. Basically, one rinse routine will consume about 15 mL of water.
30. Clean Hamilton syringe with sufficient water.
31. To store the sample cell dry: withdraw residual water from the sample cell using clean Hamilton syringe, treat sample cell **twice** with 350μL of ethanol and remove the solvent completely. Air blow dry the sample cell for at least 1 minute.
32. Air blow dry Hamilton syringe.

33. If you have more than one sample in the same buffer, you may clean the sample cell manually by your buffer. Please note that after this process, there will be residual buffer in the sample cell, therefore, samples may be diluted slightly. In addition, some users found it more challenging to fill a "wet" sample cell.
34. **If a concentration greater than 1mg/mL of protein is used in the sample cell,** at least 3 more times of buffer rinse is needed. In addition, 100 mL of water is required to pass through sample cell using ThermoVac. Please refer to the figure on the right.

35. **If your sample precipitates in the sample cell**, use **Detergent Soak and Rinse (Long)** routine according to the User's Manual Section 4 to clean the sample cell. In addition, after detergent soak, please wash the sample cell with 100mL water using ThermoVac and run a water to water titration to make sure the detergent is removed. No detergent is allowed passing through Washing Module.

Shut Down:

36. Empty ddH_2O vial (bottle) and waste bottle.
37. Make sure the injector syringe, Hamilton syringe and sample cell are **DRY**.
38. Close the control program.
39. Copy raw data to your directory and/or share disk.
40. Switch off the instrument.
41. Switch off the control PC.
42. File instrument log book.
43. If you plan to use the instrument **the next day**, you may fill the sample cell with ddH_2O and leave the instrument on overnight.

Data Analysis:

44. Please refer to the **ITC Data Analysis in Origin® Tutorial Guide** from MicroCal. The tutorials are clear and helpful.
45. Alternatively, use **MicroCal PEAQ-ITC Analysis Software** for analysis.