TA Instruments Affinity ITC (Megalab)

First version: 06/11/2020

This is a protocol to be used as a check list every time you run an ITC experiment. You must read this document in its entirety before you operate the instrument. You must be trained **before** you run any experiments. If you have any specific questions about the instrument or how to troubleshoot problems, please reach out to **Adalberto Díaz (Chazin Lab)** before you change anything in the hardware, or you attempt to do any manual cleanings.

Protocol: How to use the Affinity ITC

1. The computer is already **ON**. If the computer is **OFF**, proceed to turn it **ON** before the following steps.
2. Turn **ON** the Affinity ITC instrument. The **ON/OFF switch** is located at the back of the instrument, immediately adjacent to the power cord. If you find the instrument ON, you may omit this step (**Note**: The computer is already **ON**)
3. Wait for **five minutes** after you turn ON the instrument. This wait time is necessary to ensure that Microsoft Windows has recognized the USB connection input from the Affinity ITC.
4. Enter your **VU username** and **password** to get access to Microsoft Windows Desktop.
5. Open the ITC program by double-clicking the icon **"ITCRun"**.
6. In the **"Experimental Method"** tab, enter **25** in the **Temperature Set Point** and click **Update**.

Syringe Cleaning Procedure

7. Click the **"Syringe Clean Method"** tab.
8. In **Solution Source**, select **Solvent 2.** (Note: Solvent 2 corresponds to the **2% Contrad** cleaning solution)
9. In **Approximate volume**, enter 10.
10. Click **Add Step to Clean Method**.
11. In **Solution Source**, select **Solvent 1.** (Note: Solvent 1 corresponds to the **deionized H_2O**)

Note: Solvent 1 and Solvent 2 lines are already connected to the deionized H_2O and 2% Contrad bottles, respectively.

12. In **Approximate volume**, enter 30.
13. Click **Add Step to Clean Method**.
14. Repeat steps **11** and **12**. At this point, you should have the following cleaning method:

Step	Source	Approximate Volume (mL)	Total Time (s)
1	Solvent 2	10	182
2	Solvent 1	30	336
3	Solvent 1	30	336

15. Click the **Black Triangle (Play)** icon located at the right side of the table to start the cleaning process.

Sample Cell Cleaning Procedure

16. Click the Flashlight icon to **turn on** the light that illuminates the cell area.
17. Remove the deionized H_2O from the sample cell using a **long needle syringe**.
18. Connect the line that goes to the Vacuum flask in the **upper end** of the **Cleaning tool**.
19. Connect the line that goes to the One-liter bottle of **filtered deionized H_2O** in the **side end** of the **Cleaning tool**.
20. Carefully insert the **Cleaning tool** into the sample cell.

Warning: Always be careful when you insert the syringe or the cleaning tool into the cell to avoid any scratch in the surface of the cell.

21. In the Cleaning station, click **Pump**.
22. Let the sample cell be washed with ~1 L of **filtered deionized H_2O**.
23. To stop the vacuum, click **Pump** again on the Cleaning Station.

Filling the Reference Cell

24. Remove **the cap** form the reference cell using the **forceps** available for the ITC.
25. Remove the deionized H_2O from the reference cell using a **long needle syringe**.
26. Load the reference cell with 300 – 350 µL of **filtered and degassed deionized H_2O**.
27. Remove the deionized H_2O
28. Repeat **steps 26 and 27** two more times.
29. After the cleaning step, add 300 µL of **filtered and degassed deionized H_2O**.
30. Using the forceps, insert the cap in the reference cell.

Running an experiment

Note: As an example, the EDTA and $CaCl_2$ will be used as solution standards to validate ITC measurements.

Injection Syringe (Titrant)	0.95 mM $CaCl_2$ in 10 mM MES pH 6
Sample Cell	0.15 mM EDTA in 10 mM MES pH 6
Reference Cell	Deionized H_2O
Buffer	10 mM MES pH 6

31. All the solutions need to be **filtered and then degassed** for at least 10 min in the degassed station before their use.
32. The reference cell already contains **the deionized H_2O (see step 28). Do not add buffer in the reference cell.**
33. Remove the deionized H_2O from the sample cell using a **long needle syringe**.
34. Load the sample cell with 300 – 350 µL of **10 mM MES (pH 6) buffer.**
35. Remove the buffer from the sample cell.
36. Repeat **steps 34 and 35** two more times.
37. Add 300 µL of **0.15 mM EDTA in 10 mM MES (pH 6)** into the sample cell.
38. Click the Flashlight icon to **turn off** the light that illuminate the cell area.
39. Enter the following parameters under the **"Experimental Method"** tab.

Stirring rate (rpm)	125
Temperature Set Point (°C)	25
Peak Height to Width Ratio	Medium
Auto Save Experiment	Save data every **10** minutes
Syringe concentration (mM)	0.95
Cell concentration (mM)	0.15
	Select **Incremental Titration**
Total number of injections	24
Minimum injection interval (s)	200
Maximum injection interval (s)	250
Volume of injection (μL)	2

Note: You can use these parameters as a starting point for your first experiment.

40. To fill the titrant (Syringe) with the $CaCl_2$ solution, click the **Green Triangle (Play)** button. **The Manual Syringe Load Wizard** will open to guide you step by step how to load the sample. These steps are shown below:
   - **a.** Select **"Refill Syringe"** and click "Next".
   - **b.** Load 350 μL of the buffer into **short needle syringe.**
   - **c.** Remove the short needle and replace it with the **adapter tool**.
   - **d.** Attach the **loading syringe** (the syringe with the adapter tool) to the **injection assembly** and load 300 μL buffer.
   - **e.** Leave the loading syringe connected in the injection syringe and click "Next" in the Wizard.
   - **f.** After **step d**, load the Injection Syringe with ~500 μL of **air**.
   - **g.** Leave the loading syringe connected in the injection syringe and click "Next" in the Wizard.
   - **h.** Then, load the injection syringe with 150 μL of **0.95 mM $CaCl_2$ in 10 mM MES (pH 6).**
   - **i.** Leave the loading syringe connected in the injection syringe and enter the volume **150** in the Wizard. Then, click next
   - j. Remove the loading syringe and reconnect the fitting to the ITC syringe fill port.
   - k. Click Finish to start the experiment.
   - l. The injection syringe will move automatically from its station to the sample cell.

<u>After the experiment:</u>

41. To clean the injection syringe, follow **steps 7 to 15** of this protocol.
42. The injection syringe will move from the sample cell back to its station to perform the cleaning step.
43. To clean the sample cell, remove the sample from the sample cell using the long needle syringe.
44. Add 300 μL of 2% Contrad into the sample cell and then remove it from the sample cell. **<u>Do not add Contrad into the reference cell</u>**.
45. Add 300 μL of deionized H_2O into the sample cell and then remove it from the sample cell.

46. Follow steps 18 – 23 of this protocol.
47. Remove the deionized H_2O from the sample cell
48. Add 300 μL of deionized H_2O to the sample cell. (**Note:** the sample and the reference cells need to be stored with deionized H_2O).
49. Put the orange cap to cover the cells area.
50. Clean the syringes with deionized H_2O (3 – 5 times). Stored all the items in their boxes.
51. Click **"Exit"** in the ITCRun program.
52. **Logout** from your account in Windows. **Do not shut down the computer**
53. Turn **OFF** the Affinity ITC instrument following step 2.

Instructional Manual:

For more information about the instrument or how to set-up an experiment in the Affinity ITC, you can access the **Affinity ITC Instruction Manual** which is available in pdf format in the **ITCRun program**.