Running Isothermal Titration Calorimetry (ITC) Experiments

Last updated: 06/05/2016

This is a quick reference manual to be used as a checkpoint list every time you run an ITC experiment. This manual is specifically written to guide the use of our Auto Affinity ITC instrument (TA Instruments). The instrument is fully automated and requires no manual inputs except for the filling and emptying of the reference cell and receptor and titrant sample plates. You must read this document in its entirety and turn in a signed copy to **PI** Netz Arroyo before you operate the instrument. You must be trained by a senior member of our lab BEFORE you run any experiments. If you have any specific questions about the instrument or how to troubleshoot problems, please reach out to **PI** Netz Arroyo before you change anything in the hardware, or you attempt to do any manual cleanings.

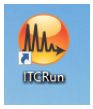
Step-by-Step Description of How to Operate the Instrument

Before Loading Your Samples

[1] The first step is to turn on both the Affinity ITC and its autosampler. The ON/OFF switches are located at the back of each unit, immediately adjacent to the power cords. If you find the instruments ON, you may omit this step.

[2] Wait for five minutes after you turn ON the two instruments. This wait time is necessary to ensure that Microsoft Windows has recognized the USB connection inputs from the Affinity ITC and Autosampler.

[3] Next, open the Run ITC program by finding the icon on Microsoft Desktop and double clicking on it.



After the program starts, you will hear mechanical noises from the instrument. The noises are normal and indicate that the computer can effectively send commands to the autosampler and that the instrument responds to those commands.

[4] Before you do anything with the instrument, make sure the arms are parked. If in doubt, you can click the “park arms” button in ITC Run. The icon looks like this:

[5] Once the arms are parked, the next step is to fill the reference chamber with reference solution. **This is the same electrolyte you are using for the titration experiments, without ligand or titrant**. You must rinse the reference cell three times with reference solution before filling it, the fourth time, with the reference solution you will use in the experiment. The reference cell must be filled with 300-500 µL of reference solution. To fill the cell, you must first turn on the LED light from the software by clicking on the icon:

Then, proceed to remove the cell plug (black plug on the right of the cell) with the forceps provided with the instrument, located on the polystyrene support above the autosampler. Next, you must **1)** rinse the cell and fill it with your reference sample, **2)** place the black plug back into the reference cell, and **3)** turn OFF the LED light.

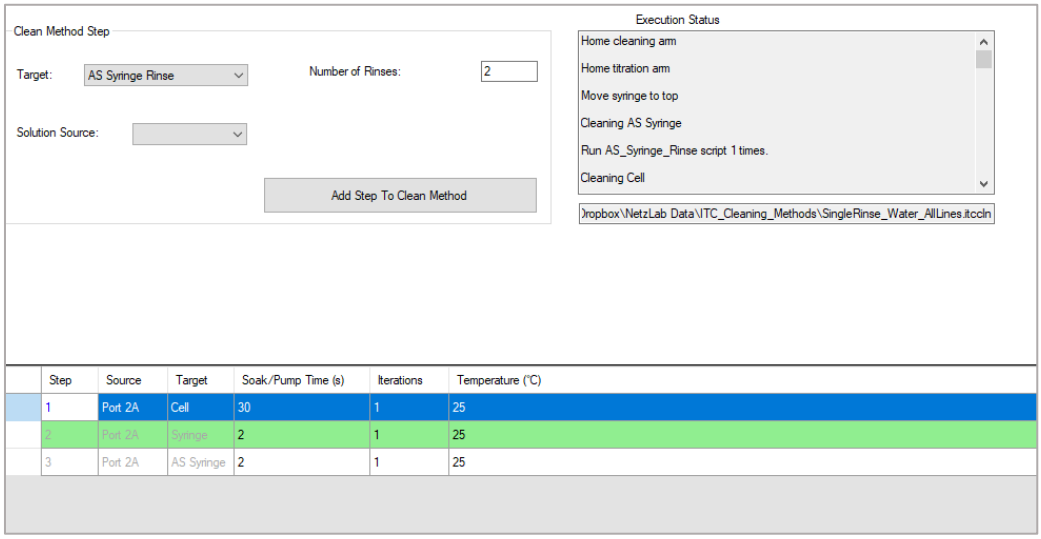
[6] Reminder: the reference cell must be filled with 300-500 µL of the same background electrolyte you are using in your titration experiment. When placing the filling needle into the reference cell, *the needle must softly touch the bottom of the cell, but must not hit the bottom of the cell with too much impact. Please avoid bending the needle.*

Creating a Cleaning Method

Our instrument is programmed to run predefined cleaning protocols before the first and in-between experiments. **PI** Netz Arroyo and other previous users have established cleaning protocols located in the PC folder:

C:\Users\NetzLab_3\Netz Lab Dropbox\NetzLab Data\ITC_Cleaning_Methods

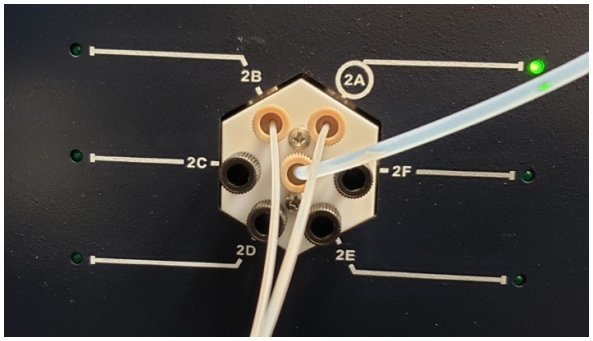
If the titration experiment you are trying to run requires specialized cleaning (if your samples are not aqueous, or involve some nasty, gooey, or sticky chemicals), Please talk to **PI** Netz Arroyo before you run them in the instrument. To create a new cleaning method, you must click on the corresponding tab ("Clean Method Step") where you will find the following window:



The following definitions apply:

Target: Identifies the target of the cleaning step. This can be the sample cell, the syringe, or the injection lines. All these targets should be cleaned prior to running an experiment to ensure that the system is clean.

Solution Source: Identifies which entry port is used for the cleaning step. Our system comes with 6 entry ports that can be connected to different cleaning solutions.

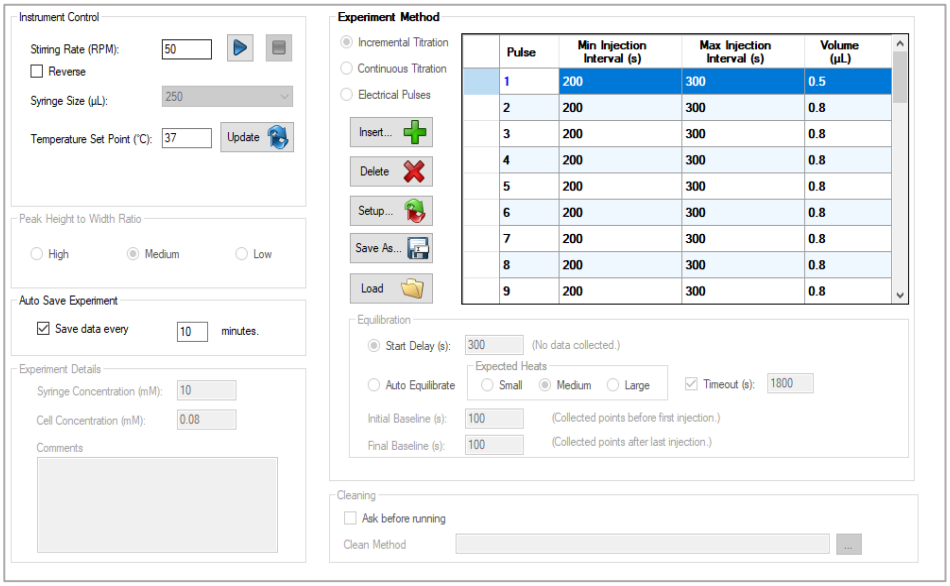


Note: *if multiple solutions are being used (e.g., mild to harsh detergents) make sure that a DI water cleaning step is used both before and after each rinse (ex.* Milli-Q H_20 → SDS → NaOH → Milli-Q H_20).

Number of Rinses: Determines number of repetitions of each cleaning step. ***Note:*** If temperature is a variable in the experiment, make sure to input the **SAME** temperature for each step.

Creating an Experimental Method

The experimental method is a set of instructions given to the instrument so it performs the ITC titration. The setup window looks as follows:



Stirring Rate: The speed of solution mixing. In our experience, the range of 50 – 100 RPMs is optimal for most titrations. Stirring at faster speeds can create extremely long injection tails in the heat charts; slower stirring can produce broad heat peaks.

Syringe Size: **Our syringe size is 250 µL**. This is the maximum volume you can inject during a titration experiment.

Temperature Set Point: For a standard ITC titration this input should be set to 25ºC. **Note:** The temperature of the method must match the temperature of the autosampler tray and of each of the cleaning steps. Otherwise the instrument will heat and cool (a lengthy process) in between method steps. When you change the temperature, click on the "Update" button to transfer the temperature input to the pulse list.

Experiment Method: The method can be set to be an incremental titration, a continuous titration and electrical pulses. This manual only concerns incremental titrations. You can setup a new pulse list by clicking on the "Setup…" button. Doing this will allow you to define the number of injection pulses, wait intervals, and injection volumes. To add or delete a step, you may click on the "Insert" or "Delete" buttons, respectively. You may also modify the steps by individually clicking on each from the pulse list. The following reference parameters are important:

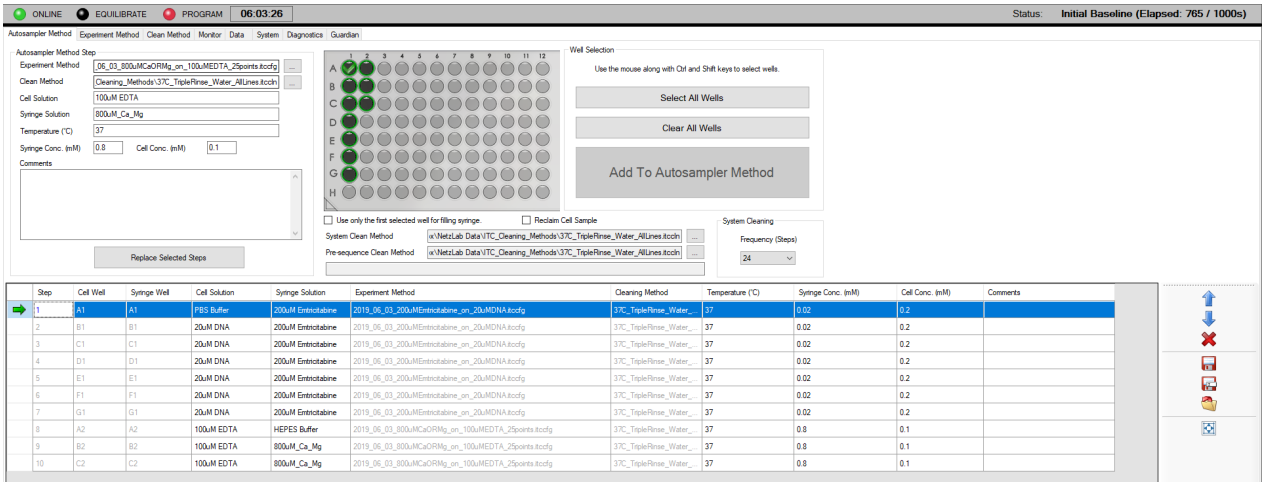
- We usually set the minimum injection interval to 200 s.
- The maximum injection interval typically should not be shorter than 250 s.
- The injection volume has a minimum input value of 0.49 uL; however, you should never aim to inject less than 0.4% the maximum capacity of the syringe. So, for our 250 µL syringe, ideal injection volumes are at ≥ 1 µL.

Equilibration: We recommend “Start Delay” time of 500 s, followed by an “Initial Baseline” of 1000 s and a final baseline of 500 s.

Make sure you save your Experimental and Cleaning Methods for future use.

Creating an Autosampler Method

The Autosampler Method defines the experiments to be performed by the instrument on each sample well. For each sample in any given run, you must define the Experiment and Cleaning Methods to be used and fill in the information corresponding to the run (concentrations, temperature, solutions, etc.). After you have established the Autosampler Method you want to run, add steps by selecting the sample wells to be used by the autosampler. This is illustrated in the image below:



Once your autosampler method has been defined, you can read the total volume needed for both the syringe and the sample cell in each run (as well as the total volume of cleaning solutions) by clicking on the flask icon located in the tool bar:

Preparing Samples for ITC Run

Based on the volumes calculated in your Autosampler Method, you can proceed to prepare the solutions to be used in your experiment. For small receptors (e.g., EDTA), a good starting concentration is 100 µM. For larger receptors (e.g., DNA, peptides or proteins), concentrations of 20 µM are a good starting point. The concentration of the titrant should be ~10x higher than the concentration of the receptor. Make sure to degas your solutions before placing samples in the autosampler tray.

Acknowledgment of Standard Operating Procedure Guidelines and Policies

I certify that I have read and understood the contents of this document and that I will abide by all the policies and guidelines described in it.

Member Name	Signature	Date