

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 <u>turn in a signed copy</u> to **PI** Netz Arroyo before you operate the instrument. You must be trained by a senior member of our lab <u>BEFORE</u> 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:

-Clean Method Step Target: AS Syringe Rinse ∨ Number of Rinses: 2 Solution Source: ∨ Add Step To Clean Method						2 thod	Execution Status Home cleaning am Home titration am Move syringe to top Cleaning AS Syringe Run AS_Syringe_Rinse script 1 times. Cleaning Cell Iropbox\NetzLab Data\ITC_Cleaning_Methods\SingleRinse_Water_AllLines.tccln	
	Step	Source	Target	Soak/Pump Time (s)	Iterations	Temperature (°C)		
	1	Port 2A	Cell	30	1	25		
	2			2	1	25		
						25		
						05		

The following definitions apply:

<u>*Target:*</u> 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.

<u>Solution Source</u>: 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 $H_20 \rightarrow SDS \rightarrow NaOH \rightarrow Milli-Q H_20$).

<u>Number of Rinses</u>: 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:

Instrument Control	Experiment Method					
Stirring Rate (RPM): 50	Incremental Titration	Pulse	Min Injection Interval (s)	Max Injection Interval (s)	Volume (µL)	
Reverse	Continuous Titration	1	200	300	0.5	
Syringe Size (µL): 250 V	Electrical Pulses	2	200	300	0.8	
Temperature Set Point (°C): 37 Update 🚯	Insert	3	200	300	0.8	
	Delete 💙	4	200	300	0.8	
	Delete	5	200	300	0.8	
Peak Height to Width Ratio	Setup 🔞	6	200	300	0.8	
	Save As	7	200	300	0.8	
High Medium Low		8	200	300	0.8	
Auto Save Experiment	Load 🖄	9	200	300	0.8	
	Equilibration					
	Start Delay (s): 30	00 (No	data collected.)			
Experiment Details	Auto Equilibrate	Expected Heats	Medium 🔿 Large	✓ Timeout (s): 1800)	
	Initial Paseline (a):	00	(Collected points before fi	tiniaction)		
Cell Concentration (mM): 0.08	Initial Daseine (s).	00	(Collected points after last	injection.)		
Comments	Final Baseline (s):	JU	(concercer points enter real	injocuori.)		
	Cleaning					
	Ask before running					
	Clean Method					

<u>Stirring Rate</u>: 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.

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

<u>Temperature Set Point</u>: For a standard ITC titration this input should be set to 25°C. **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.

<u>Experiment Method</u>: 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.

Standard Operating Procedure



<u>Equilibration</u>: 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:

0	ONLINE		IBRATE 🥥 F	ROGRAM 06:0	3:26						Status:	Initial Baseline (Elap	sed: 765 / 1000s)
Antosempler Method Begenerert Method Ocen Method Nontor Data System Dagrostica Guardan													
-Auto Eq	Advaseping Method Sep Exementer Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 00, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 000M-MCAGNMBg, on, 100M-MEDTA, Zignents Actor) - A Queen Method (56, 000M-MCAGNMBg, on, 100M-MCAGNMBg, on, 100M-MG, o												
Cel	Ceaning_inethods/3/C_Inpenning_inethods/3/C_I						Select All	Wells					
Te	Syringe Solution 800uM_Ca_Mg Temperature (*C) 37				D		Clear All						
Co	Sympe Conc. Initial (0.8) Cel Conc. Initial (0.1) Comments					Add To Autosampler Method							
Replace Skieteld Steps Pre-sequence Dean Method (w/NetLub Data/17C_Dearing_Methods/37C_TripleRine_Water_Millines.toch) = 24 v													
	Step C	Cel Wel	Syringe Well	Cell Solution	Syringe Solution	Experiment Method	Cleaning Method	Temperature (°C)	Syringe Conc. (mM)	Cell Conc. (mM)	Comments		4
-	1 A	a –	A1	PBS Buffer	200uM Emtricitabine	2019_06_03_200uMEntricitabine_on_20uMDNA.tocfg	37C_TripleRinse_Water	37	0.02	0.2			
	2 B		81	20uM DNA	200uM Emtricitabine	2019_06_03_200uMEntricitabine_on_20uMDNA.tocfg	37C_TripleRinse_Water	37	0.02	0.2			*
	3 C		C1	20uM DNA	200uM Emtricitabine	2019_06_03_200uMEntricitabine_on_20uMDNA.tocfg	37C_TripleRinse_Water	37	0.02	0.2			×
	4 D		D1	20uM DNA	200uM Emtricitabine	2019_06_03_200u/MEntricitabine_on_20u/MDNA.toofg	37C_TripleRinse_Water	37	0.02	0.2			
	5 E		E1	20uM DNA	200uM Emtricitabine	2019_06_03_200uMEntricitabine_on_20uMDNA.tocfg	37C_TripleRinse_Water	37	0.02	0.2			
	6 F	1		20uM DNA	200uM Emtricitabine	2019_06_03_200uMEntricitabine_on_20uMDNA.tccfg	37C_TripleRinse_Water	37	0.02	0.2			
	7 G			20uM DNA	200uM Entricitabine	2019_06_03_200u/MEntricitabine_on_20u/MDNA.tccfg	37C_TripleRinse_Water	37	0.02	0.2			<u> </u>
	8 A		A2	100uM EDTA	HEPES Buffer	2019_06_03_800uMCaORMg_on_100uMEDTA_25points.tccfg	37C_TrpleRinse_Water	37	0.8	0.1			Ø
	9 B		82	100uM EDTA	800uM_Ca_Mg	2019_06_03_800uMCaORMg_on_100uMEDTA_25points.tccfg	37C_TripleRinse_Water	37	0.8	0.1			
	10 C			100uM EDTA	800uM_Ca_Mg	2019_06_03_800uMCaORMg_on_100uMEDTA_25points.tccfg	37C_TripleRinse_Water	37	0.8	0.1			

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 μ M. For larger receptors (e.g., DNA, peptides or proteins), concentrations of 20 μ M 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.