TA Instruments ITCRun™ Software Getting Started Guide

Instrument Types Supported

ITCRun software is used to control the operation of the Nano ITC III, Nano ITC Standard Volume, and Nano ITC Low Volume isothermal calorimeters. The calorimeter model name appears on the instrument front panel and in the **Setup** tab of ITCRun while it is connected and online. The labels on Nano ITC Standard Volume instruments that shipped before September 2009 read "Nano ITC^{2G}." Standard and low volume instruments incorporate second generation technology featuring enhanced baseline stability and increased sensitivity.

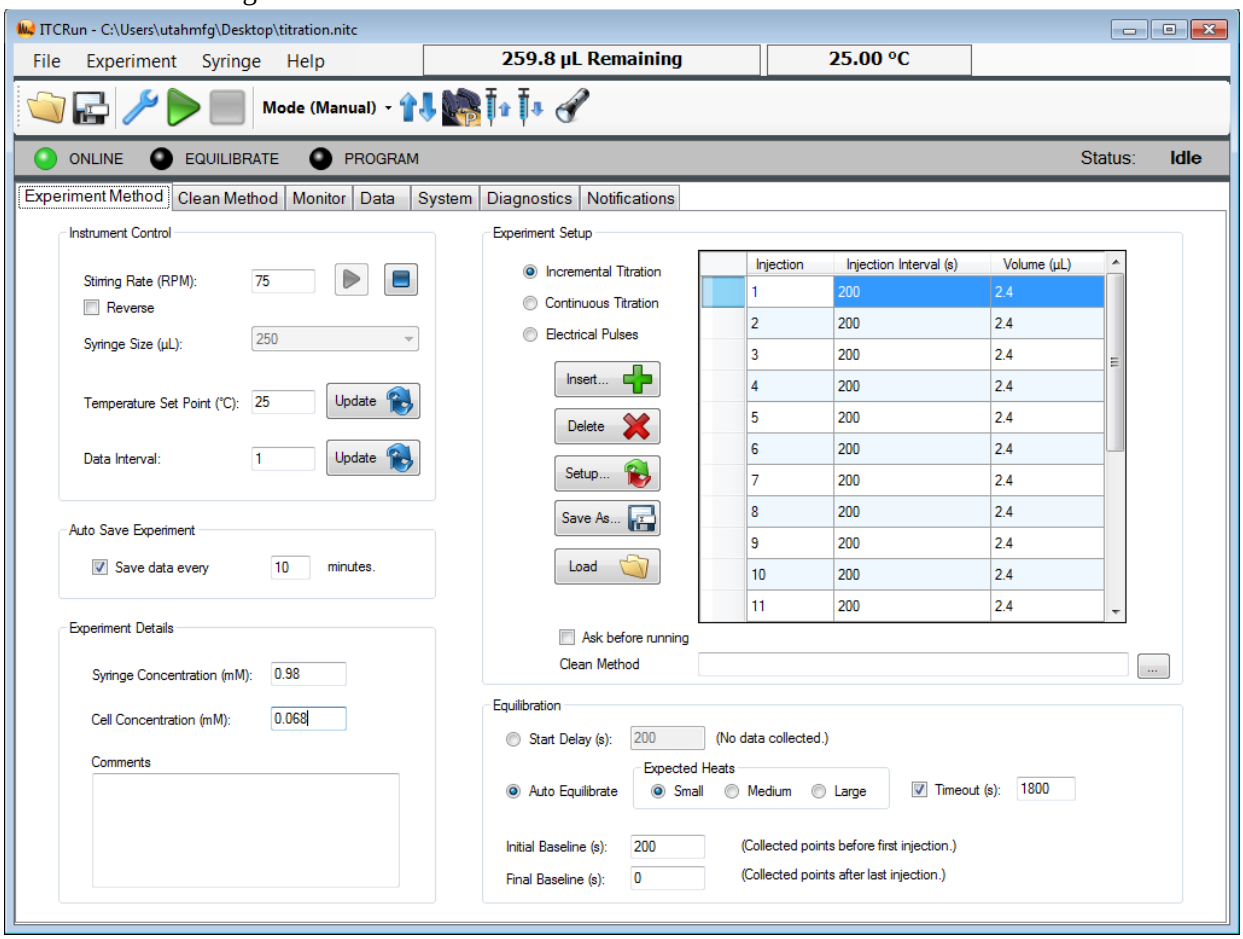
Getting Ready

1. Install the ITCRun software and the instrument drivers before connecting the Nano ITC instrument to the computer.
2. Connect one end of the power cable to the instrument and the other end to a power outlet.
3. Connect a USB cable between the instrument and the computer.
4. Set the power switch (located at the rear panel of the instrument) to **On**. A green light appears on the front window of the instrument.
5. The PC may display a message that it has found new hardware and is looking for the driver. Select **No, not at this time** if you are asked to search for the driver via the internet, and select the option to install the driver automatically.

Note for IT personnel: Since the data control and collection software depends on accurate timings, it is highly recommended to set the computer BIOS settings for performance rather than for power savings. Some computer manufacturers may have different names for this. For example, some Dell computer have a setting called "C-States" that includes the C1E setting, which should be disabled. Other computer manufacturers may call it "Enhanced Halt State". Other settings that should be disabled (if available) are EIST (Intel SpeedStep) and AMD's Cool 'n' Quiet. Microsoft Windows® should be set for **Performance Mode** instead of **Low Power Mode**. In addition, Windows Update should be set to avoid automatic reboots of the computer.

Starting ITCRun Software

1 Double-click the **ITCRun** desktop icon or select the **ITCRun** shortcut in the Windows Start menu. The program opens a window on the desktop. A bright green “online” indicator indicates the software is communicating with the instrument. .



2 The following functions are located on the **Menu** bar:

- **File**: Open data file, Save data file, Exit program
- **View**: Shows or hides the toolbar and status bar
- **Experiment**: Instrument Settings, Start or Stop an Experiment, Electrical Calibration
- **Buret**: Moves the syringe drive Up or Down
- **Help**: Access Program Help, connect to Software Download Web page

3 The following functions are located on the toolbar:

- Open File 
- Save File 
- Instrument Settings 
- Start Experiment (data collection) 
- Stop Experiment (icon activates when running an experiment) 
- Raise buret plunger 
- Lower buret plunger 
- Move syringe to defined position 
- Reset the home position of the buret 

Setup Tab

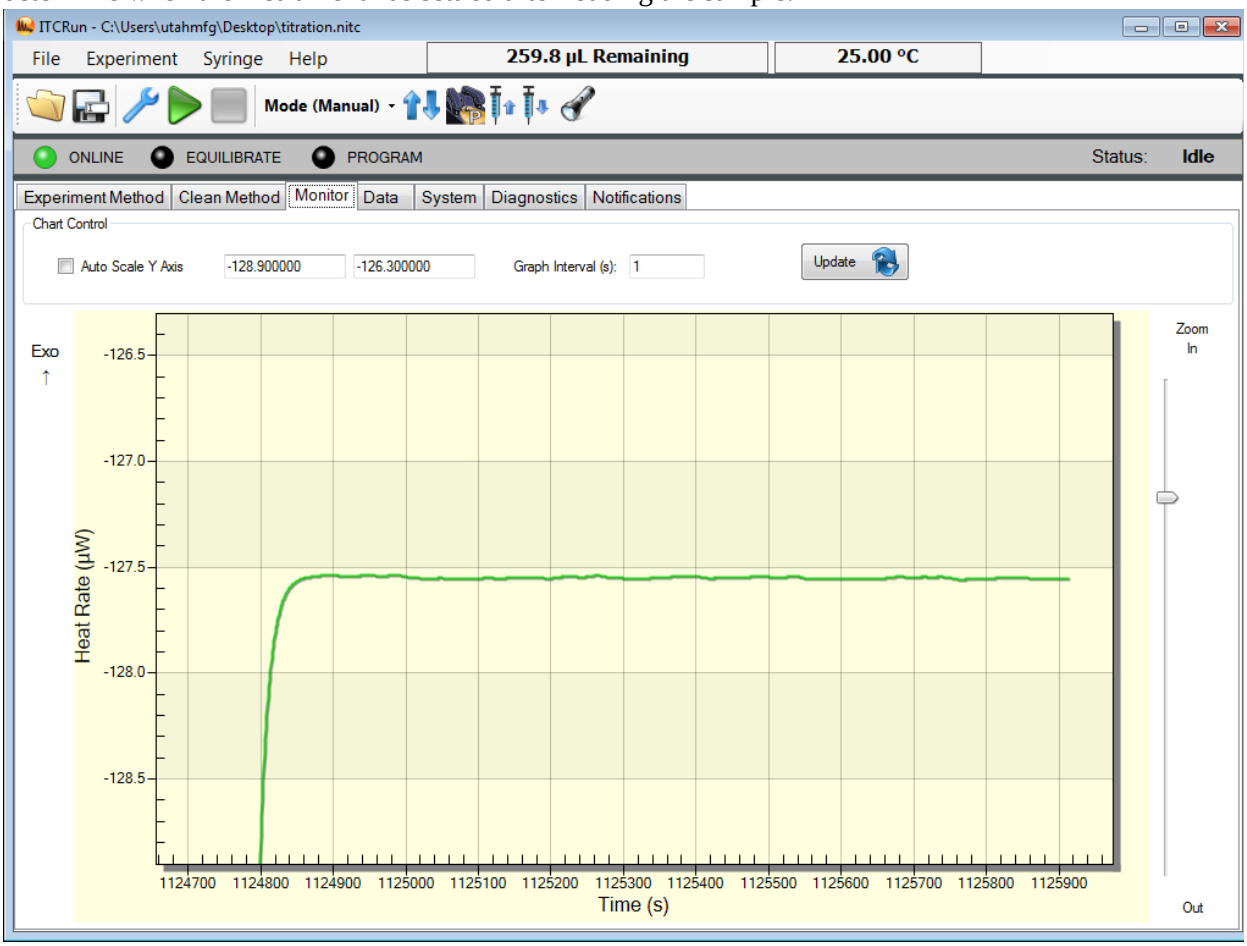
The following functions are located on the **Setup** tab.

- **Instrument Status indicators and controls**
  - Sample temperature
  - System signals including temperature-controlled zones and sample cell data
  - Running time
  - Online status (green if the instrument is connected and operating, gray if not)
  - Equilibration indicator (yellow during the time before the experiment if the start delay or automatic equilibration features are in use)
  - Program (red when an experiment is in progress, gray if not)
- **Stirring rate setting**
  - The stirring rate can be set between 150–400 rpm. Suggested stirring rates are 250-350 rpm for Standard Volume (gold), 350 rpm for Low Volume, and 150–200 rpm in instruments with Hastelloy® cells. Slow mixing rates of the sample may require a faster speed and/or a longer injection interval. Higher stirring speeds may increase the baseline noise.

- **Stirring Start and Stop controls**
  - These buttons start and stop the stirring motor. After inserting a syringe, another few minutes will be needed for the final settling. The settling will progress slightly faster if the syringe is inserted gradually and left partially inserted for a few minutes (one inch or 2 to 3 cm short of full insertion) before the final full insertion. This allows the syringe needle to equilibrate with the temperature control zone but does not exchange heat directly with the sample cell. Stirring should typically be running during experiments to allow the reactants to mix.
- **Syringe Size selection**
  - Set this field to match the syringe that is currently loaded into the buret. The Nano ITC Low Volume instrument is compatible with the 50 µL syringe only. The Standard Volume instruments are compatible with both the 100 and 250 µL syringes. Do not attempt to insert these larger syringes into the Low Volume instrument.
- **Experiment Type selection**
  - Select incremental titration for discrete injection events at timed intervals, continuous titration for one gradual injection occurring over a single timed interval, or electrical pulses for basic system response checking.
- **Sample temperature setting**
  - The operating temperature range is 2–80°C; experiments are conducted at a single temperature for the entire run. Set the desired sample temperature for the experiment. The current sample temperature is displayed at the top of the program window. For the quickest instrument settling, pre-equilibrate the sample in a thermostat, such as the Degassing Accessory from TA Instruments. Sample temperatures that are far below or above ambient conditions may exhibit periodic noise in the baseline. If this occurs, reduce the stirring speed and use longer injection intervals.
- **Data collection rate control**
  - The data collection rate is the interval, in seconds, between saved data points. A 1 second rate is typical. Longer data collection intervals (such as 2 seconds) may be used with slowly changing signals, such as those that might be encountered during the Continuous Titration experiments.
- **Automatic data file save control**
  - Use this control to save a copy of the experiment data at the specified interval. If the run is accidentally interrupted due to a power failure, all the data collected up to the most recent save event will be preserved.
- **Injection/Calibration pulse control table**
  - These settings define the experiment schedule.

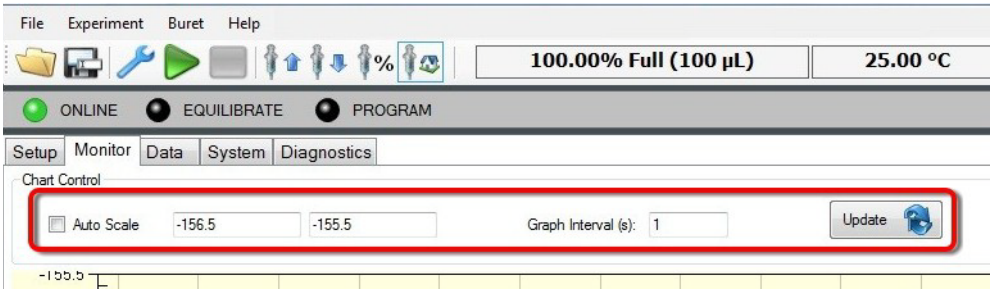
Monitor Tab

Select the **Monitor** tab at the upper left corner of the ITCRun program window. Use this screen to determine when the instrument has settled after loading the sample.



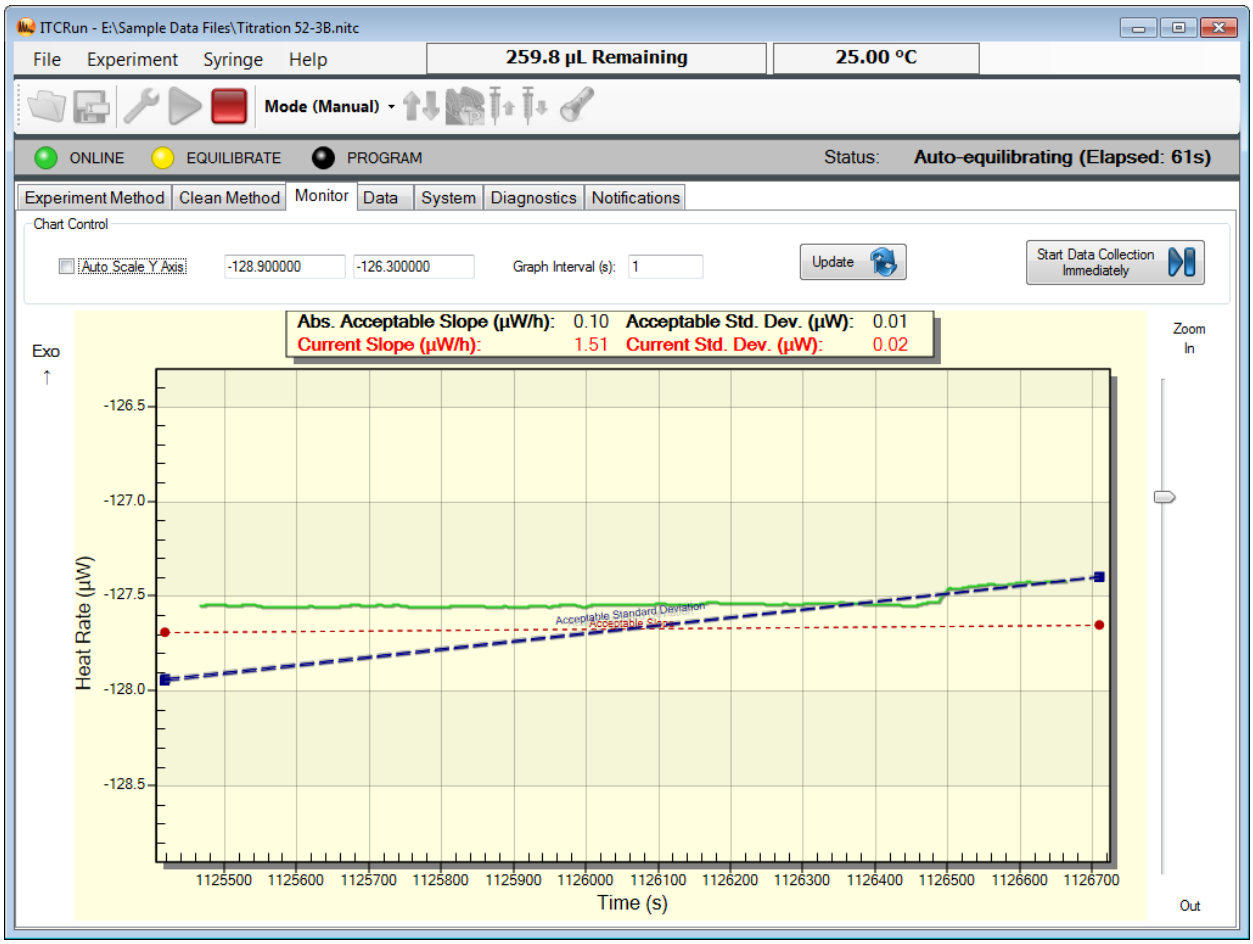
- **Chart Controls**
  - **Update**: Applies the manually entered minimum and maximum values for the Y-axis.
  - **Auto Scale** check box: The heat signal trace will expand to the full height of the chart when selected; deselect in order to use the manual scaling controls.
  - **Manual Vertical Movement** (click and drag mouse icon within graph window): Adjusts the Y-axis up and down.

- Manual scaling is possible when the **Auto** checkbox is unselected. Set the desired upper and lower vertical limits of the display, then click **Update**. A vertical axis range of 1 µWatt is very convenient for watching a baseline to stabilize before starting an experiment. When the baseline is flat to within a range of 0.02 to 0.03 µWatts, it should be easy to accurately integrate even very small peaks.



- **Equilibration Settings**: Several options automatically start an experiment after the baseline settles.
  - **Start Delay**: Sets a fixed time delay before starting an experiment. Use this control to allow a small amount of additional time for a baseline to settle. When this mode is used, the signal stability criteria are ignored.
  - **Auto Equilibrate**: Starts an experiment automatically when user-specified baseline stability criteria for both baseline noise and slope are satisfied (these criteria can be numerically in the **Settings** dialog box, and/or graphically modified in the **Monitor** tab).
    - **Expected Heats**: Select more stringent stability criteria of smaller enthalpies are expected in the experiment.
    - **Timeout**: Use this control to force an experiment to start after a user-chosen time interval, even if the stability criteria have not been satisfied. When samples are loaded into the instrument and stirring is active, small amounts of the solution in the syringe diffuse into the cell. Therefore, it is not possible to maintain a sample in the instrument for extremely long periods of time. This control allows the collection of the best possible data under difficult baseline conditions.

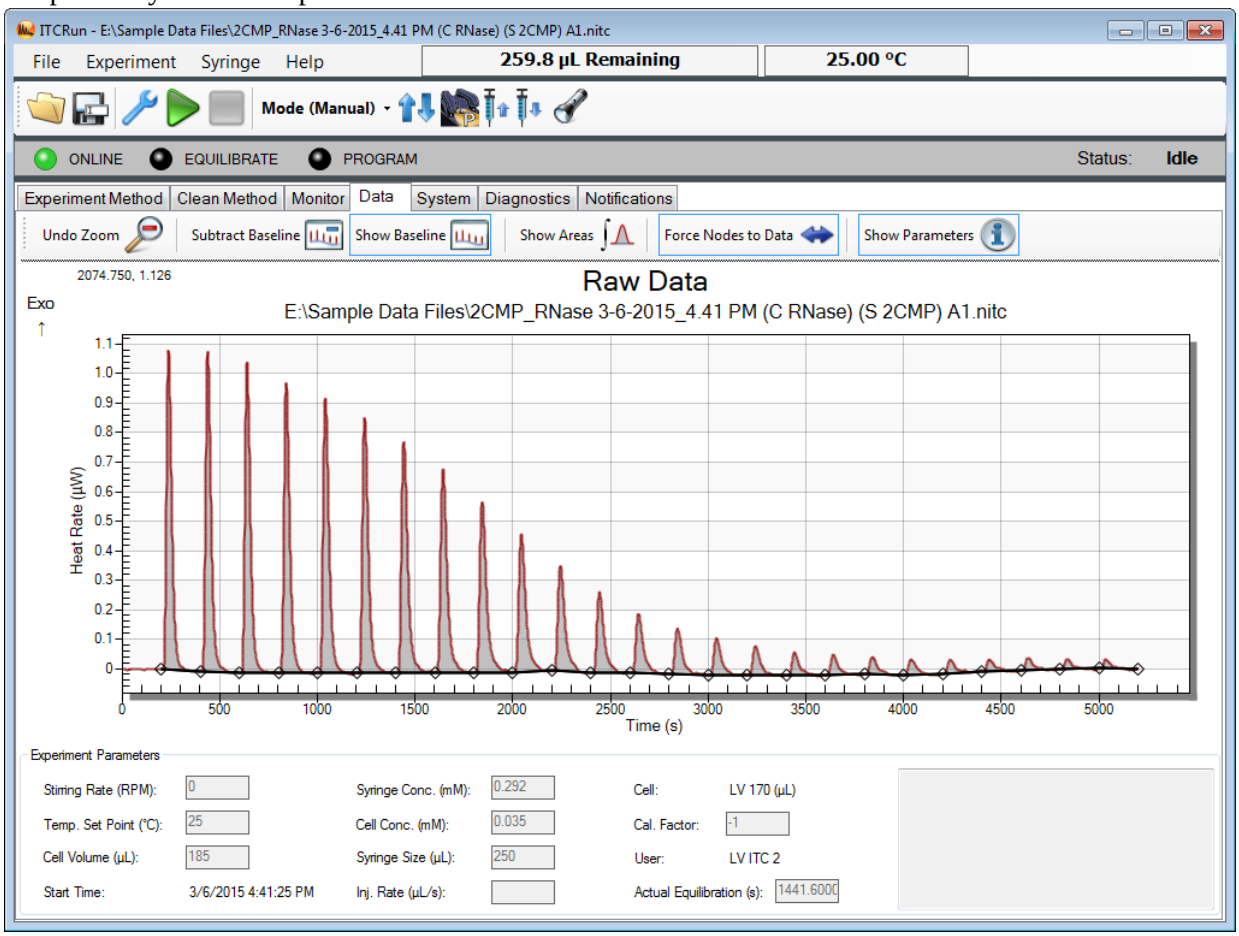
NOTE: At any time during the **Auto Equilibration** interval, the stability criteria can be modified on the **Monitor** page. Use the slider controls on the **Slope** and **Standard Deviation** indicator lines.



The equilibration criteria are continuously updated on the signal data chart in the Monitor tab.

Data Tab

This displays the heat signal while running experiments. Injection events are indicated and a preliminary baseline is drawn between injections. The data from the previous experiment remains on the screen until it is replaced by the next experiment.

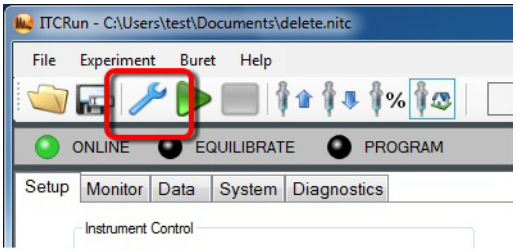


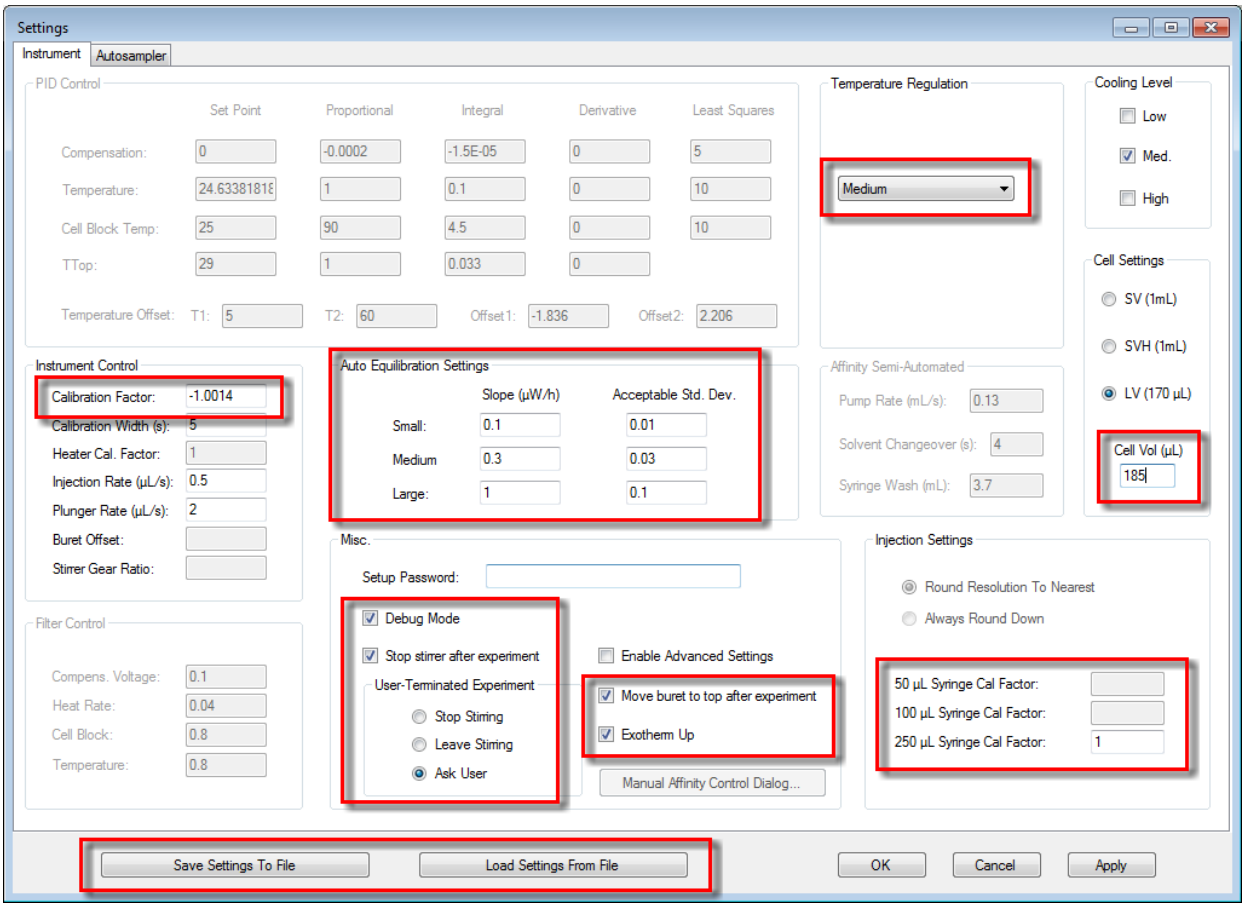
- **Toolbar items:**
  - **Undo zoom**: Rescales the display to show all available data.
  - **Subtract baseline**: Rescales data chart with baseline nodes set to zero µWatts. A preliminary integration is performed and the areas are displayed for each peak.
  - **Show baseline**: Toggles display of the baseline on and off.
  - **Show area plot**: A preliminary integration is performed. Each area is represented as a single point on the chart.
  - **Force Nodes to Data**: When moving the baseline control points manually, this forces the points to stay aligned on the data.
  - **Show Parameters**: Displays or hides the sample data.

Instrument Settings

The instrument settings window is accessible while the instrument is idle between experiments. Click **Settings** or select the **Settings** item in the **Experiment** menu. The instrument calibration factor is automatically stored upon completion of the electronic calibration and can be accessed in this window. When performing a chemical calibration, the factor would be computed externally by the operator and then manually entered into the provided entry box. Enable **Debug Mode** to show the **System** and **Diagnostics** tabs.

NOTE: To ensure that the proper control settings are used, be sure to select the correct instrument type. The settings are saved in nonvolatile memory inside the Nano ITC^{2G}. Nano ITC III settings are saved in the computer.





Common setting options available to users.

CAUTION: Critical instrument controls are set on this page. Changing these settings can cause the instrument to operate incorrectly. TA Instruments retains a backup copy of the settings for every instrument.

Analysis of titration experiments requires an accurate figure for the effective cell volume, which varies slightly from one instrument to another. For best results, a chemical calibration should be performed to establish this figure. Detailed procedures are documented in “Calibration of nanowatt isothermal titration calorimeters with overflow reaction vessels”, Neil A. Demarse, Colette F. Quinn, Dennis L. Eggett, Donald J. Russell, Lee D. Hansen, Analytical Biochemistry 417 (2011) 247-255.

If a chemical calibration of the cell volume has not yet been performed with a specific instrument, these default volumes will be fairly close:

Cell Type	Affinity ITC	Nano ITC
Standard Volume	965 microLiters	950 microLiters
Low Volume	185 microLiters	170 microLiters

Once the cell volume has been established, enter the figure in the provided entry box in the Settings tab of ITCRun. The figure will be saved in the non-volatile memory of the instrument.

If there are any doubts about correct instrument settings, the default values can be restored. Select **Load Default Settings** from the **Experiment** menu item in the main screen of ITCRun. Navigate to the **Settings** dialog box and click **OK** or **Apply**. Note that loading the default settings does not alter the existing calibration factor.

The following settings are available to the user as required:

- **Stop stirrer after experiment**
- **User-Terminated Experiment**: allows a preference of how to manage the stirrer when a user terminates an experiment manually.
- **Move buret to top after experiment**: Selects whether the buret should remain in place or reset to the top of the stroke when as experiment ends.
- **Calibration width** (>5 seconds are required for calibration pulses exceeding 1400 µJoules)

NOTE: The Cell Block Temperature Control box should be selected when operating the Nano ITC instrument.

- **Auto Equilibration Settings** adjust according to the typical baseline noise characteristics for the experiment.
- The **Calibration Factor** field adjusts the signal gain after having run a Chemical Calibration.
- Select the **Debug Mode** checkbox if you wish to display the **System** tab.

Selecting **Debug Mode** enables a fourth screen tab titled **System**. Four instrument information channels display on the **Settings** window, with the following default choices selected:

- **Nano ITC III**

Temperature:	Cell Block DAC
Cell Block Delta V:	Compensation

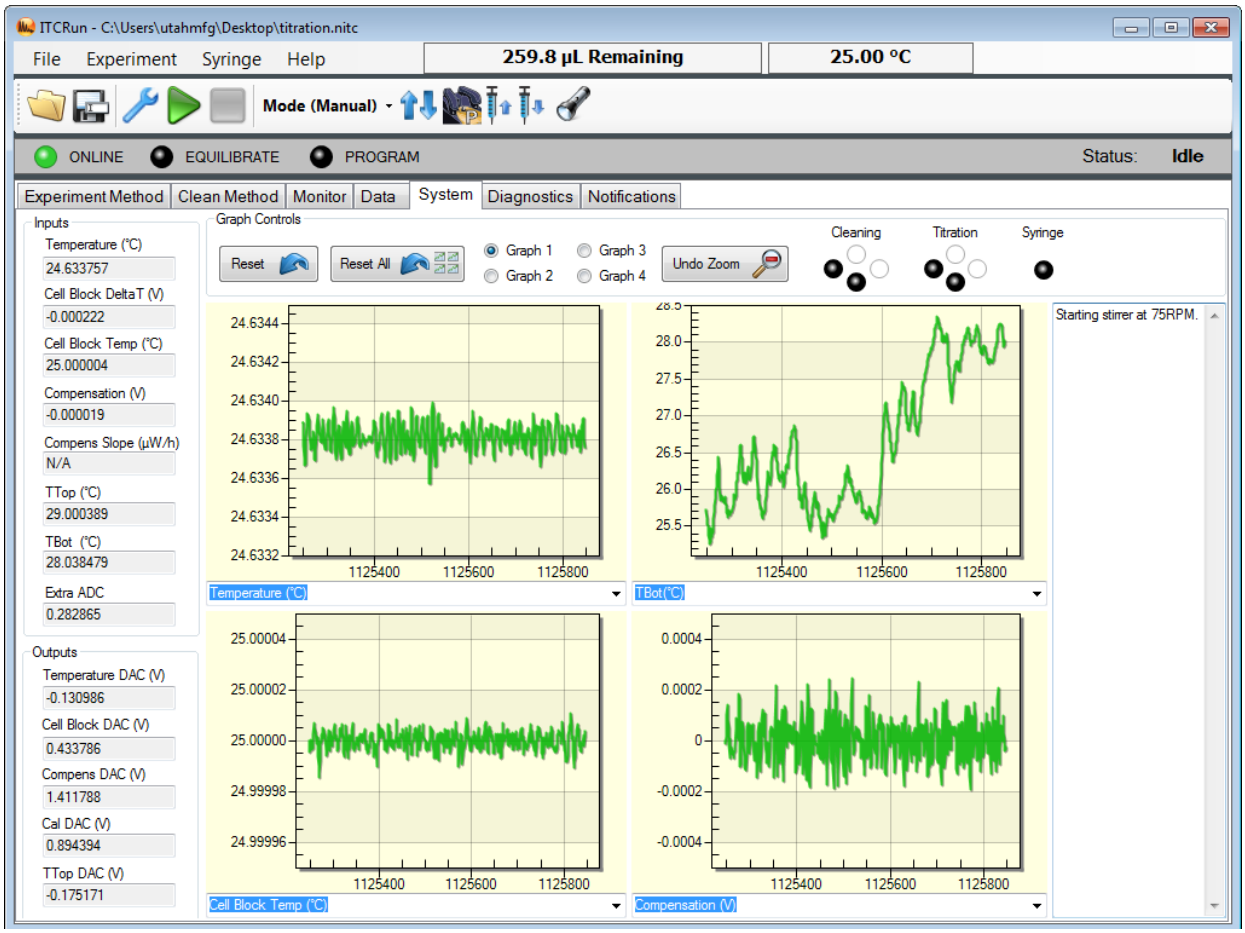
- **Nano ITC Standard Volume and Nano ITC Low Volume**

Temperature	Cell Block DAC
Cell Block Temperature	Compensation

Select **OK** to accept the new settings, or **Cancel** to exit without making changes.

System Tab

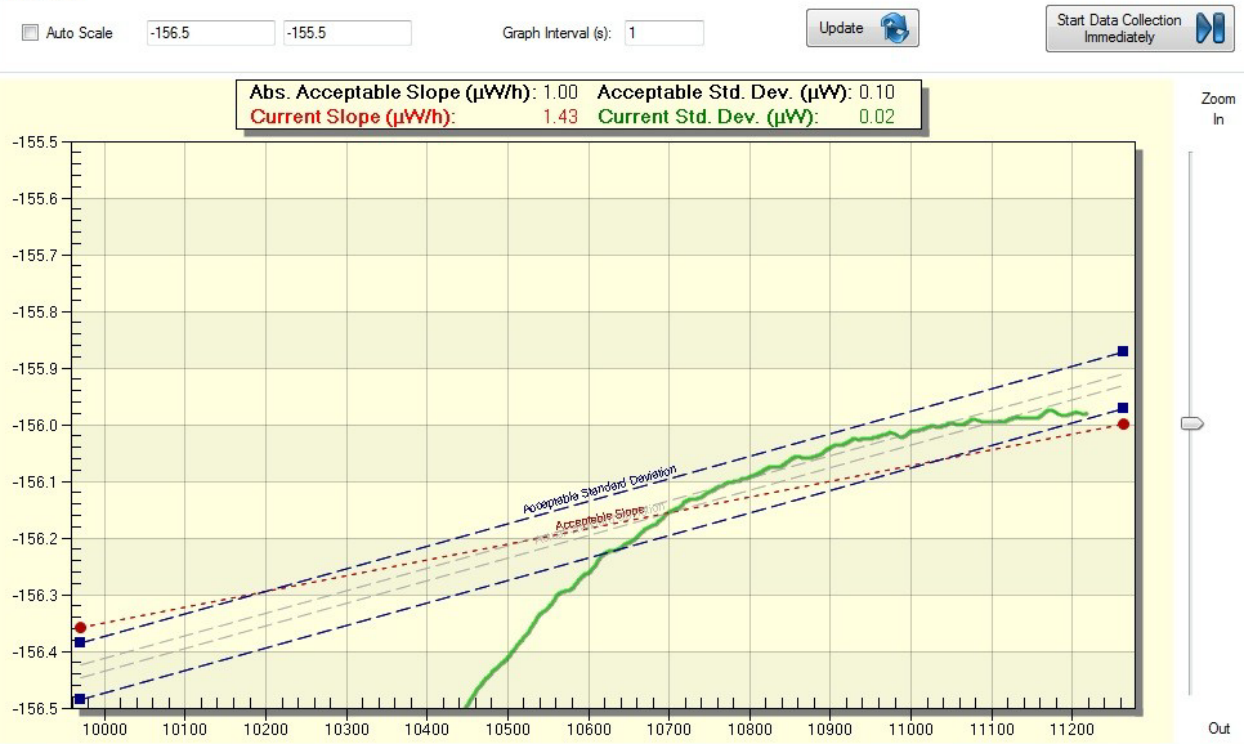
This window monitors the internal instrument parameters. It can be used to check on the progress of the thermal stabilization to see if an experiment can be started. After a cold start, the temperature zones will be moving towards their set points. The charts act as short-term monitor windows and do not record data. If this tab is not visible in the program window, it can be enabled by selecting **Debug Mode** in the **Settings** page.



When a sample is introduced into the measurement cell, it adjusts to the instrument temperature. The graphs on the **System** tab display the status of the temperature-controlled zones of the instrument. These zones become stabilized first, followed by the heat signal which can be viewed on the **Monitor** tab.

The primary data signal that is generated by the ITC is the rate of heat flow into or out of the sample cell. The status of this signal is continuously charted in the **Monitor** tab. When a sample is loaded, the heat signal typically goes through a temporary full-scale excursion. This is caused by even minor temperature differences between the sample and the instrument.

Before manually starting an experiment, use the **Monitor** tab to determine when the instrument has settled sufficiently. To ensure accurate integration of titration data, the signal baseline should be free of curvature and display a low noise level and drift rate. Peak-to-peak signal excursions should be less than 0.1 µWatts. A bent injection syringe needle or altered instrument control settings can cause the noise level to exceed that figure. The baseline drift should generally be allowed to settle below 0.1 µWatts over a period of several minutes before an experiment is started. When the experimental enthalpies are expected to be very small, take special care to achieve a very quiet and flat baseline.



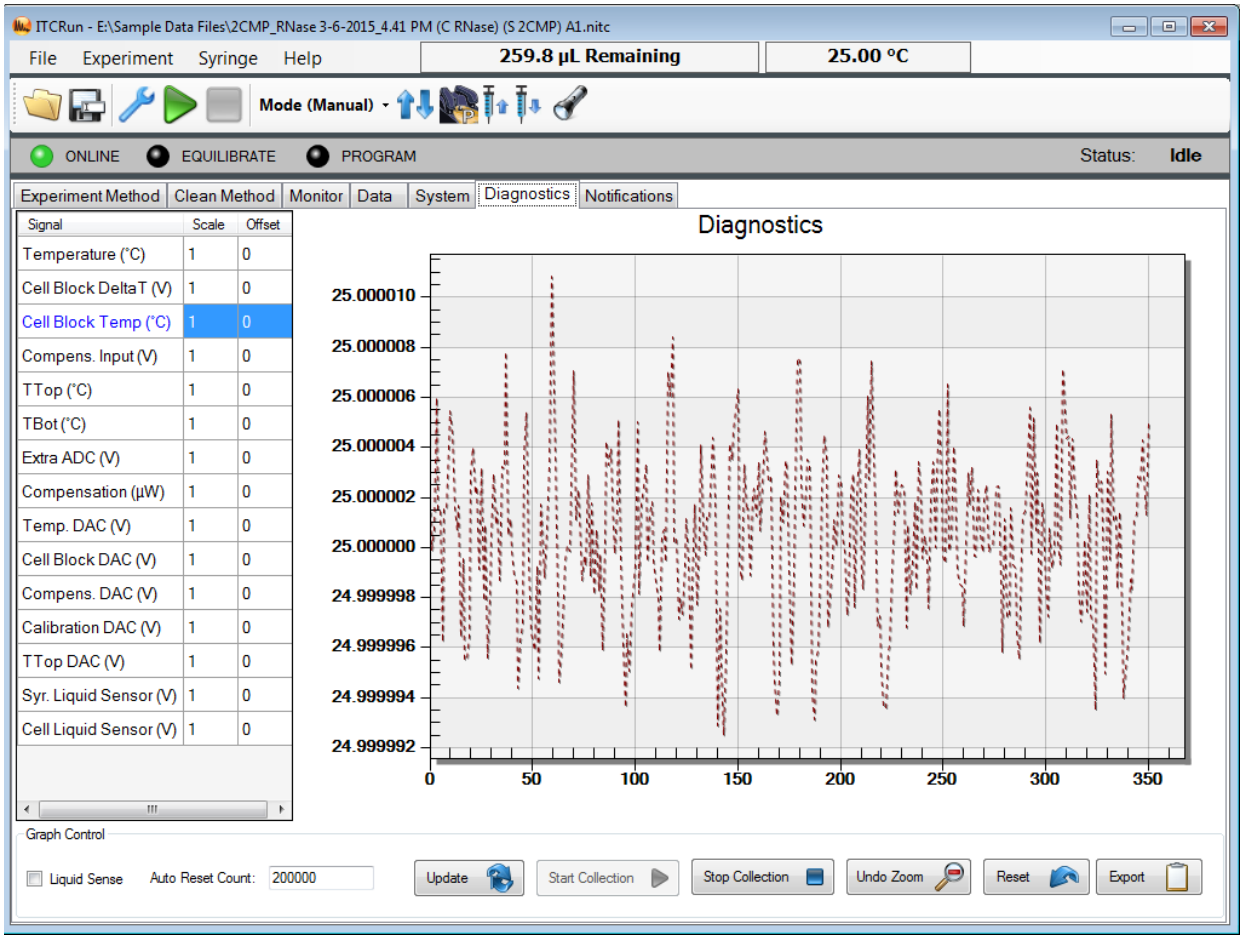
The **Auto Equilibrate** feature can be used to automatically determine when a baseline signal has sufficiently stabilized to provide high-quality experimental results. The signal must be settled according to two criteria: the noise level and the slope of the baseline signal. The most recent 10 minutes of signal are continuously evaluated. The slope and standard deviation are computed and updated on the graph with a pair of gray lines. The user-set criteria for signal noise is superimposed as a pair of blue lines. The square buttons at the end of the lines are control handles that can be dragged with the mouse in order to modify them to wider or narrower limits, as desired.

The slope limit is set according to the values entered in the **Settings** dialog box but can also be altered at any time by dragging the control buttons. The absolute value of the current slope is compared against the absolute value of the user-set slope limit. Therefore, it does not matter if either slope is positive or negative.

As soon as both criteria are satisfied, the experiment will begin.

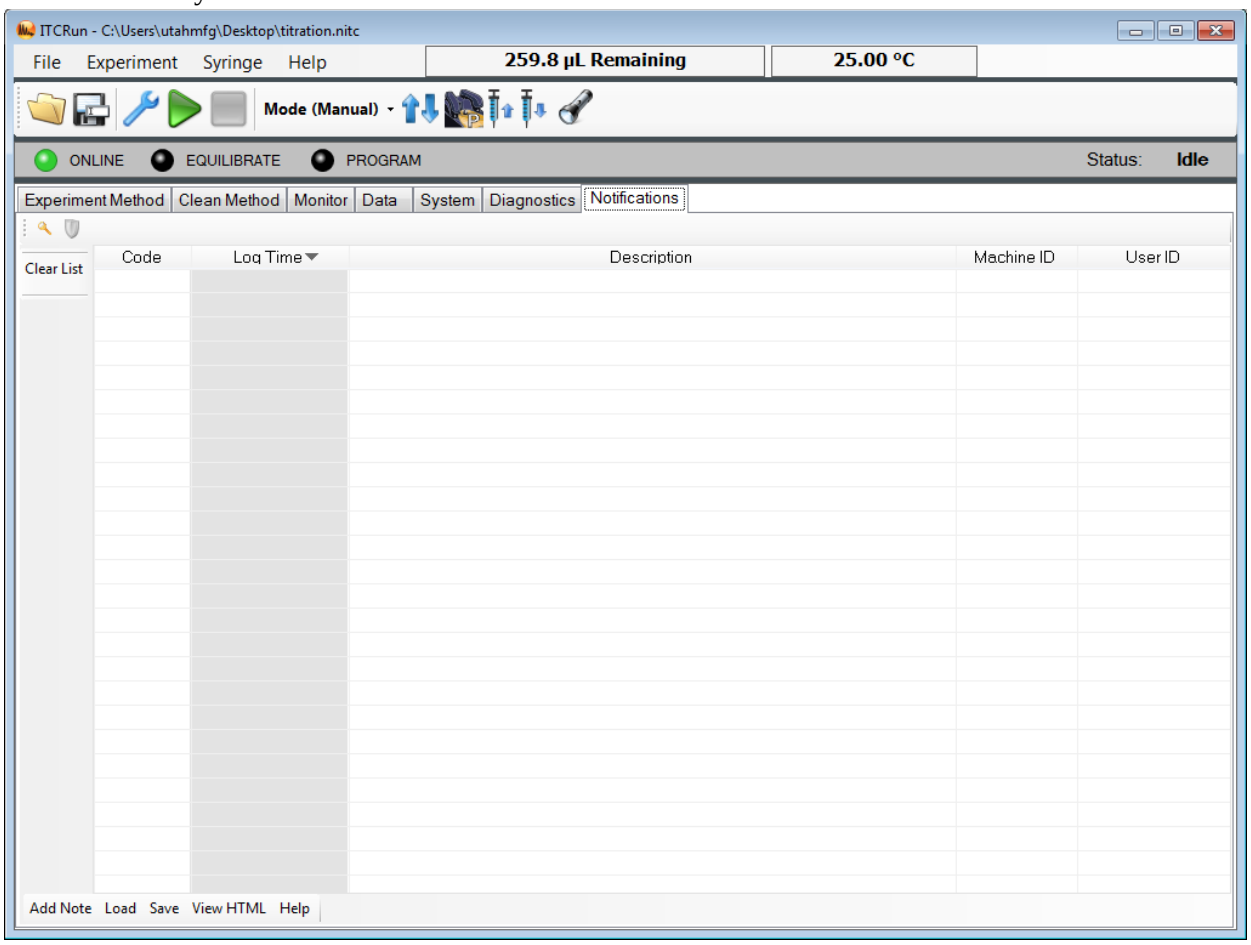
Diagnostics Tab

The **Diagnostics** tab can be used to observe up to two internal parameters for longer periods of time. It operates differently from the **Monitor** tab in that the data does not disappear out of the window. It can be used for long-term monitoring of the instrument operation. Select the channel(s) of interest and click **Start Collection**. The chart can be cleared and restarted by selecting **Reset**. **Stop Collection** stops and clears the chart window. If this tab is not visible in the program window, it can be enabled by selecting **Debug Mode** in the Settings page.



Guardian Option

The Notifications tab is where Guardian option features reside. Guardian is an implementation of 21 CFR Part 11 regulations. Detailed information about Guardian is available in the document “Guardian for Microcalorimetry Software.”



Experiment Overview

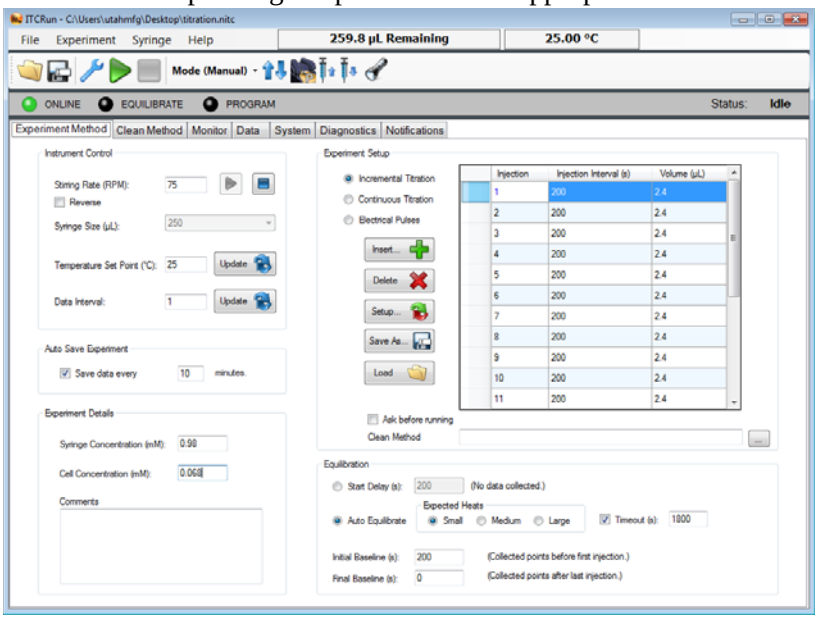
Defining an Incremental Titration Sequence

1 Select 250 or 350 rpm stirring speed for initial trial runs in the case of gold cell instruments (use 150 or 200 rpm for Hastelloy).

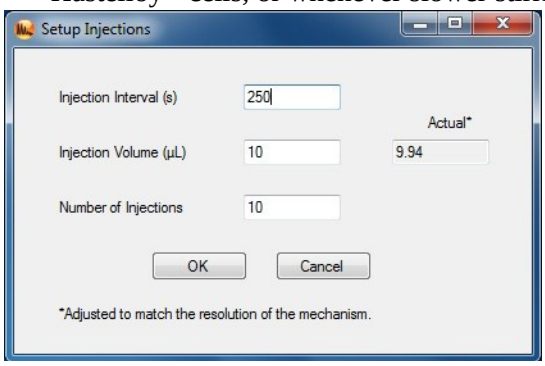
2 On the **Setup** tab, select **Incremental Titration** in the **Experiment type** window.

3 Select the appropriate syringe size. The 100 or 250 µL syringes can be selected with the Nano ITC Standard Volume instrument. The Nano ITC Low Volume instrument uses only the 50 µL syringe.

4 Select the operating temperature that is appropriate to the reaction being studied.

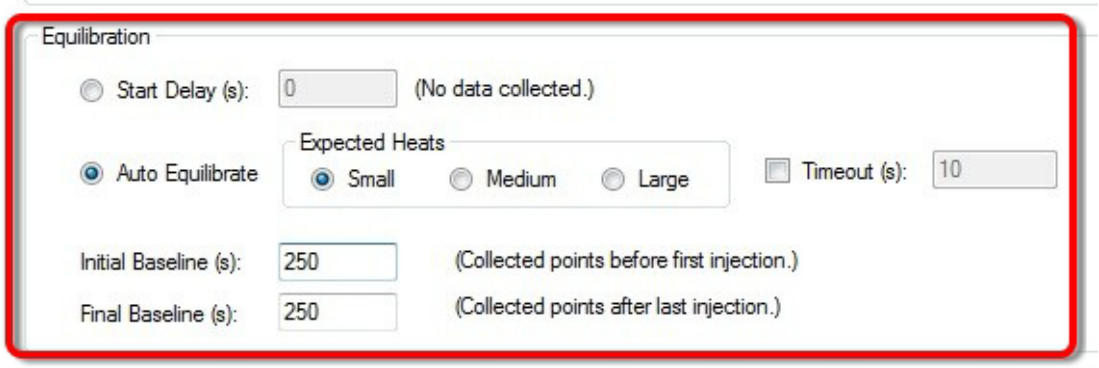


5 Select **Setup** to define an injection table. For instruments with gold cells, select intervals of 250 to 300 seconds along with a stirring speed of 250 rpm or greater. Use longer intervals for instruments with Hastelloy® cells, or whenever slower stirring speeds are used.



NOTE: Injection volumes are adjustable in increments of 0.114% of the total syringe capacity. The actual volume will be adjusted automatically by the software as necessary in order to match the nearest available quantity that is supported by the buret mechanism. The adjusted volume is displayed to the right of the entry box and will be recorded in the data file.

6 Select the **Initial Baseline** time to an interval that is sufficient to observe the starting baseline. Setting this to the same duration as the injection interval is a good starting point.



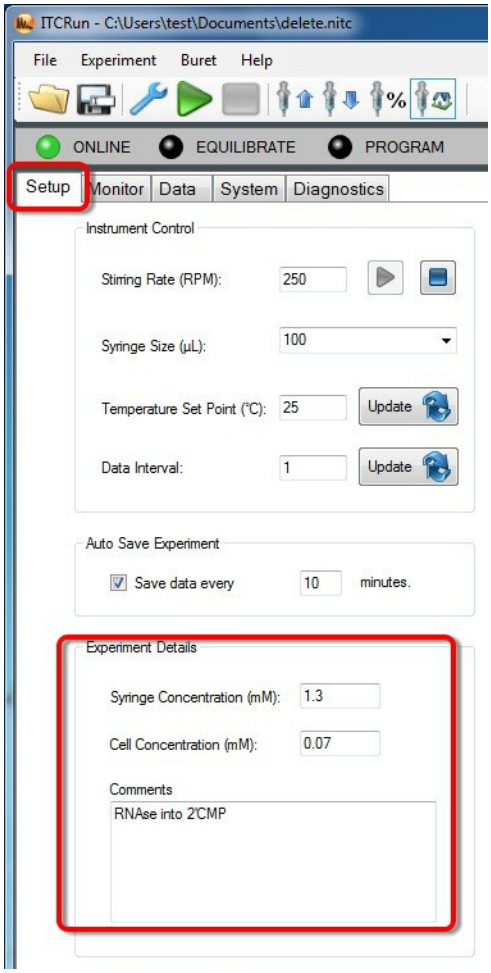
7 Refer to the *ITC Getting Started Guide* for sample loading procedures.

8 Manual Start: When the instrument stabilizes after sample loading and starting of the stirring motor, select the green **Go** button to start the experiment. The experiment can be initiated with a delayed start time in order to allow more time for the system to stabilize. Data is not collected during this interval. Enter the desired delay interval in seconds into the field labeled **Start Delay**.

Note that the sample can diffuse out of the tip of the syringe, especially in the case of small molecules. Avoid excessively long start delays; in most cases the sample loss will not be excessive with delays of up to several hundred seconds.

9 Automatic Start: Use the **Auto Equilibrate** option to have the experiment start automatically when the baseline has been stabilized.

NOTE: Starting with ITCRun software version 1.7.0, there are new data entry fields (shown below) for the sample concentrations, as well as a generic comment field that can be used for various purposes such as recording the temperature, the stirrer rpm, and the names of the reactants. This additional information is available in the NanoAnalyze software during data analysis.


ITCRun - C:\Users\test\Documents\delete.nitc
File
Experiment
Buret
Help
ONLINE
EQUILIBRATE
PROGRAM
Setup
Monitor
Data
System
Diagnostics
Instrument Control
Stirring Rate (RPM):
250
Syringe Size (µL):
100
Temperature Set Point (°C):
25
Update
Data Interval:
1
Update
Auto Save Experiment
Save data every
10
minutes.
Experiment Details
Syringe Concentration (mM):
1.3
Cell Concentration (mM):
0.07
Comments
RNAse into 2'CMP

Defining a Continuous Titration Experiment

Continuous titrations are set up in a similar manner to incremental titrations, with the following differences:

1 Select **Continuous Titration** as the **Experiment type**.

2 Set the desired injection period in the **Injection Interval** field.

- The syringe is driven at a constant rate during this interval. Note that side effects resulting from the fluid delivery will cause the baseline to shift by a small amount, typically a few µWatts. Running a blank experiment with injection of buffer into buffer is highly recommended. Every experiment detail must match the sample experiment, with the exception that the reactant species are not present.

- High enthalpy reactions may cause the heat measurement to saturate. Saturation is the condition of the heat rate at an unchanging value of either 0 or several hundred µWatts in the negative direction. The heat rate can be reduced by decreasing the injection rate (use a longer injection interval) or changing sample concentrations.

3 Set a minimum of 300 seconds in the **Final Baseline** field to allow the signal to return to the true baseline after the injection.

- The syringe is not driven during this time, but data continues to be collected. The baselines in the post-delivery interval will step into alignment with the pre-delivery interval. The true baseline goes straight through those initial and final segments.

- The step feature at the end of the syringe stroke will be present in both the sample and blank experiments, and will cancel out when subtracting the blank.

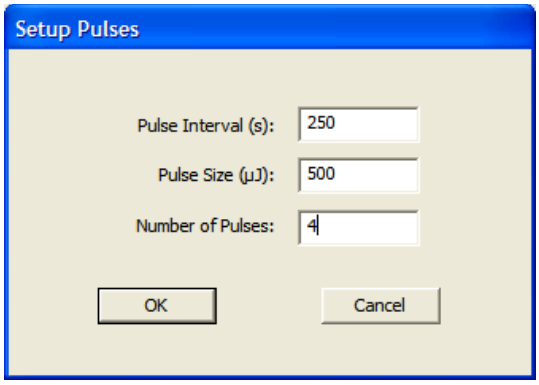
4 Continuous titration experiments should also include a blank run that is conducted with the same conditions, especially the stirring speed and time parameters. See the *NanoAnalyze Software Getting Started Guide* for instructions on subtracting the blank experiment.

Defining an Electrical Pulse Sequence

A sequence of electrically generated heat pulses can be used to verify the instrument response. Select **Electrical Pulses** in the **Experiment type** box.

Note that heat is applied to the sample cell in a slightly different location than is the case with chemically-generated heat pulses during a titration experiment. As a result, electrical pulses are somewhat taller and narrower than chemical pulses that have an equivalent energy.

1 Click **Setup** and input the desired interval in seconds, the heat pulse size in µJoules, and the number of calibration pulses, then select **OK**. The table updates when the new settings are confirmed.



2 Set the desired equilibration time in the window below the pulse table. Baseline data is collected during this time interval before the first pulse.

3 Clicking on the **GO** button (green circle) starts the experiment sequence. The sequence ends after the completion of the final pulse or when the user selects **Stop**.

Loading a Sample

Refer to the *Nano ITC Getting Started Guide* for instructions on how to load a sample. The sample cell is loaded first and allowed to stabilize before introducing the syringe. In typical ITC experiments, the Reference cell is filled with degassed deionized water. The reference needle balances the thermal characteristics of the injection needle. Place the buret (without the syringe) into place on the instrument to aid in the thermal settling. Click the **Raise Buret** control (green Up arrow) to make the buret ready to accept a full syringe.

There is a minimum sample fill volume required in order to ensure proper instrument function. Fluid should be present in the access tube to lubricate the syringe needle bushings, as well as to provide a thermal path for temperature equilibration. In addition, there is a recommended maximum fill volume which brings the fluid level to the top of the access tubes. The conical space above the top of the access tube is sufficient to receive the full capacity of the 250 µL syringe. Use fill volumes of 300–700 µL for the Nano ITC Small Volume, and 1200–1500 µL for the Nano ITC Standard Volume.

When the heat baseline appears to be flat in the **Monitor** tab, fill the syringe, load it into the buret, and install the buret and syringe into the instrument. Start the stirring motor using the checkbox in the **Setup** tab. Wait for the heat signal to stabilize on the **Monitor** tab before starting an experiment.

Starting an Experiment

Titration

Verify that degassed samples have been loaded into the cell and the syringe before proceeding. The reference cell must be filled (typically with degassed deionized water) and have the reference needle in place. There must be a small air bubble (approximately 1 mm long) at the face of the plunger tip in the injection syringe to cushion the sample delivery.

1 On the **Setup** tab, verify the following conditions:

- The proper sample temperature has been set.
- The stirring is running at the proper rpm.
- **Incremental** or **Continuous Titration** is selected and the desired injection schedule has been defined.
- The correct syringe size has been selected.

2 On the **Monitor** tab, verify that the heat signal has stabilized to within 0.2 µWatts or less in a 10- minute period. If the expected peaks will be very small, it will be best to ensure that the baseline drift is less than 0.1 µWatts over a period of 10 minutes. The time interval between red lines is 30 seconds.

- When the instrument and sample are ready, click the green **GO** button and enter the name for the data file. The user may select any file storage location such as: **My Documents \ Nano ITC Data**
- The progress of the experiment can be followed in the **Data** tab. Selected areas of the signal chart can be examined in detail by zooming in. Use the mouse to click and drag a box over the area of interest.
- The status fields at the top of the main program window indicate the current temperature, heat measurement, time, and injection number. After the completion of the first injection a preliminary baseline is drawn on the signal chart.
- At the completion of the experiment, the signal remains visible on the **Data** tab for inspection.
- It is often beneficial to perform a blank experiment in order to verify the size and uniformity of the enthalpies associated with the injection delivery. Load the syringe as in the actual experiment, but dialyzed buffer without any reactant chemical is loaded into the sample cell. Use an identical injection sequence (all timing parameters, injection size, and number of injections must match). The heat data will feature only dilution heat and friction effects. The two data files are loaded and subtracted in the NanoAnalyze program.

Electrical Calibration

1 Load degassed deionized water into the sample and reference cells and the syringe. Load the syringe and buret into the instrument. Set the stirring to 250 rpm for instruments with gold cells, and 200 rpm for Hastelloy cells.

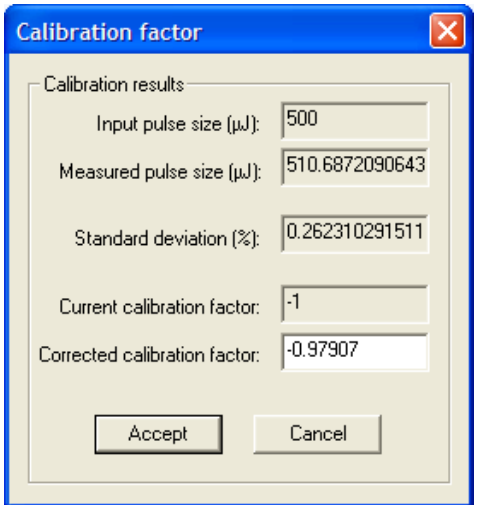
> **NOTE**: For best results, the ITC should be calibrated to a standardized chemical reaction experiment. See the Appendix for one example of a suitable system. If a calibration sample is not available, a good alternative is to perform a calibration using electrical heat pulses.

2 Set the sample temperature to the same value that will be used in experiments. Allow the temperatures to stabilize as seen in the **Monitor** tab.

3 In the **Experiment** menu item, select **Start Electrical Calibration**. The events table automatically fills in with 500 µJoule electrical pulses that require approximately one hour to complete. The pulse timing is slower in the case of instruments with Hastelloy cells. Instructions for proper preparation of the instrument are displayed.

4 After ensuring the correct conditions are met, click **OK**. A dialog box then appears which will allow the user to enter a file name for the experiment data. You may choose a name that identifies it as calibration data, and includes the date, such as "CAL Oct-28-2009". Start the calibration by clicking **Save**.

5 The experiment is started in the same way as a titration. The software asks for a name for the data file. At the completion of the calibration experiment, the program asks for the new calibration data to be accepted or canceled. Select **Cancel** if the purpose was only to check that the calibration is still valid.



TA Instruments recommends that all Nano ITC users periodically perform a calibration on their instrument using a chemical sample with known results. This calibration is important when optimum accuracy of the enthalpy (ΔH) measurement is required. If no calibration has been performed on a Nano ITC, TA Instruments recommends that an electrical calibration followed by a chemical sample calibration be performed. If a full calibration is not checked periodically, then the enthalpy results should be considered qualitative. The electrical calibration procedure will give a good approximation of the calibration constants, but the most accurate calibration will be achieved by running a chemical sample with known results. Please contact TA Applications Support for any questions on these calibration procedures.

Appendix A: Chemical Calibration

A chemical calibration tests all aspects of the instrument including the calibration constant, the cell volume, and the injection volume. Heat is released in exactly the same location as occurs during sample titrations, and therefore is the preferred calibration method. (If chemical standards are not available, electrical pulse calibrations are generally suitable as a second choice.)

There are several standard reactions which are often used in calibrating isothermal titration calorimeters (see Briggner, L.-E. and Wadsö, I. [1991] Test and Calibration Processes for Microcalorimeters, with special reference to heat conduction instruments used with aqueous systems *J. Biochem. Biophys. Methods* 22, 101-118.). Here we will describe one: protonation of Tris base (Tris[Hydroxymethyl] Aminomethane). The Tris protonation experiment may be used to determine or verify the calibration factor value setting used in the ITCRun software.

Heat of Protonation of Tris Base

Sample Preparation

It is very important to do a thorough degassing of the water to be used for making the solutions. Use the lowest ionic content water that is available, such as what is produced by a point-of-use deionized water system. Degas this water by stirring under vacuum for a minimum of 45 minutes. Do not degas the prepared solutions, because this can result in the loss of sample. The solutions can be kept for a short time in stoppered containers. Use nitrogen or argon to fill the head space in order to exclude ambient air which contains carbon dioxide.

Prepare a solution of Tris base by dissolving approximately 0.24 g in 50 mL of distilled water. The solution will be approximately 40 mM, but the exact concentration is not important as long as it is well in excess.

A 1.00 mM HCl solution is most readily prepared by pipetting 10 mL of standardized 0.1N HCl into distilled water and diluting to 1 L in a volumetric flask. Alternatively, a standard solution of HCl can be purchased commercially or standardized by acid-base titration (see Skoog, D.A. and West, D.M. [1980] *Analytical Chemistry* [Saunders College Publishing], p. 228 ff). Do not degas this solution.

Experiment Setup

Experiment Parameters

Syringe size	100 µL (50 µL in the Nano ITC Low Volume)
Equilibration time	200 seconds (Hastelloy: 300–400 seconds)
Time between injections	200 seconds (Hastelloy: 400 seconds)
Injection size	5 µL
Number of injections	20 (10 in the Nano ITC Low Volume)

1 Rinse the calorimeter cell three times with the Tris solution and then load the cell. The reference cell may be filled with degassed deionized water. Allow the cells to thermally equilibrate until the heat reading on the calorimeter is stable.

2 Load the 100 µL syringe with the 1.00 mM HCl solution, making sure to remove any bubbles from the syringe.

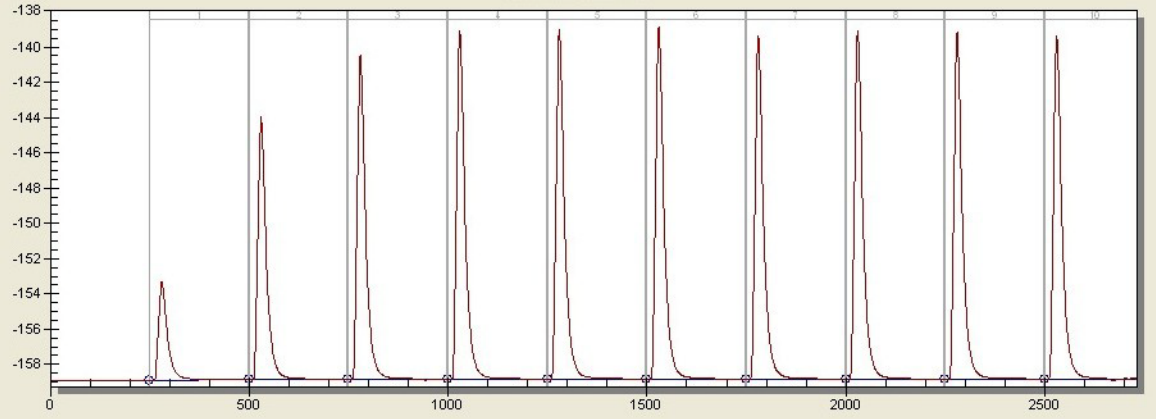
3 Wipe the needle with a tissue and then screw the syringe completely into the buret drive.

NOTE: Before inserting the syringe into the buret drive, verify that the plunger indicator on the graduated handle is in the fully raised position. Otherwise, mount the buret on the Nano ITC without a syringe and click the **Buret up** icon (green arrow pointing upwards).

4 Insert the syringe and buret drive into the Nano ITC.

5 Turn on the stirrer at 250 rpm (150 rpm for Hastelloy) and allow the system to re-equilibrate until the heat reading on the calorimeter is stable. Then begin the experiment. Enter a file name at the prompt.

The results should be similar to those shown in the figure below.



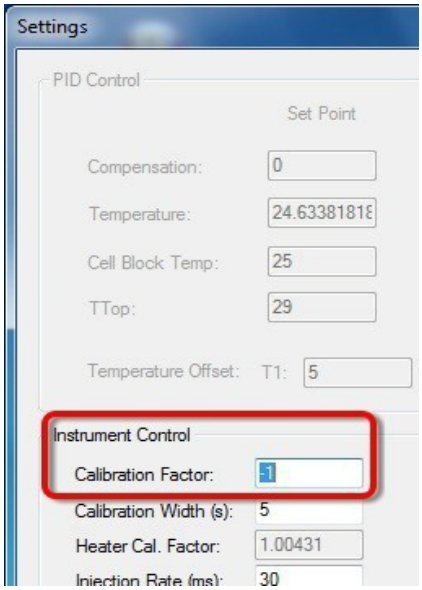
Note that each peak has the same area except for the first. Typically the first injection shows less heat than expected. This is due to diffusion through the tip of the needle, small air bubbles at the needle tip, or to differences in positioning the buret drive. For 5 µL injections of 1.00 mM HCl at 25°C, the expected enthalpy is -237 µJ. The protonation enthalpy in J/mol at any temperature between 5 and 50°C is given as:

$$\Delta H_{protonation} = -49659 + 102.28T - 0.59275T^2$$

The calibration factor (C.F.) is calculated as follows:

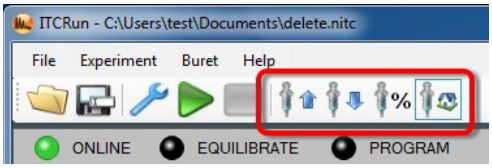
C.F. = (Expected Heat / Measured Heat) x Existing Calibration Factor

6 Calculate an average of the C.F. for several injection peaks. In the **Settings** screen (shown below), enter the new calibration factor in the provided entry box. Calibration factors for every version of the Nano ITC always has a negative sign.



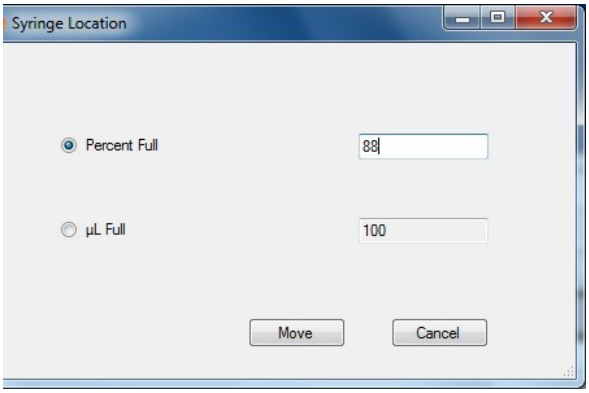
Appendix B: Buret Position Functions

ITCRun Software versions 1.8.7 and later include a new buret position reporting function. The buret can be preset to any position from 0 to 100% of the syringe capacity. The positioning can be performed either via a percent of the full stroke or directly in microliters.

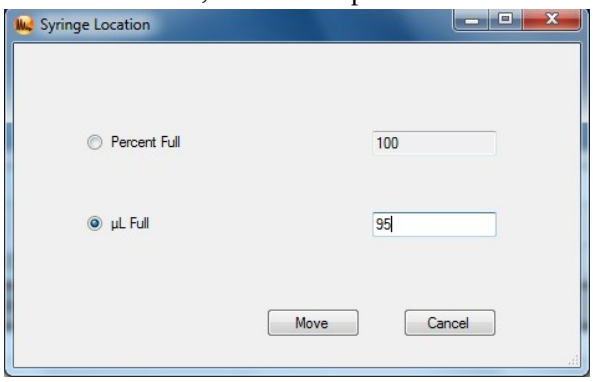


Click **Home Reset**:

The desired buret position can be entered directly in µL. (In the case if the Standard Volume ITC, be sure to set the correct syringe capacity in the **Setup** tab). Enter the desired position in terms of µL and click **Move**. The buret position field at the top of the ITCRun screen updates with the current position of the buret drive.



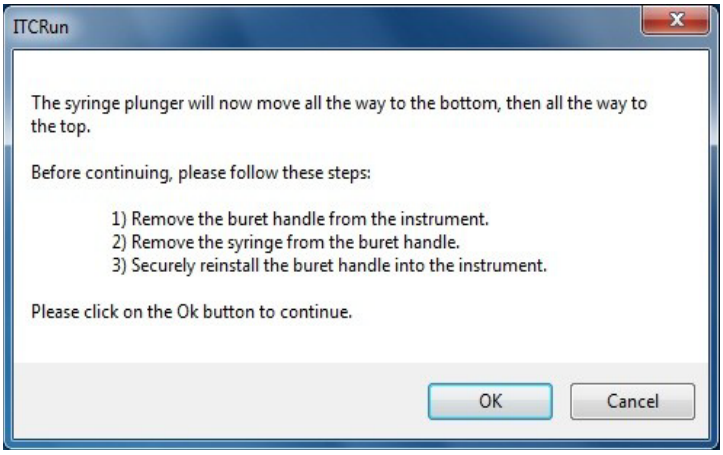
As an alternative, the desired position can be entered as a percent of the full capacity.



In order to ensure that the reported position of the buret drive matches the actual position, a new **Home Reset** function has been added. This moves the buret drive all the way to the bottom of the stroke in order to find a home reference, then moves the drive upwards by exactly the length of the syringe stroke. Follow the instructions on the screen, paying particular attention to removing the syringe from the buret handle before proceeding.

Click **Home Reset** to start the **Home Reset** process:

If the syringe is present in the buret drive and it contains a sample, note that the motions will empty the syringe. To avoid losing the sample, remove the buret handle from the NanoITC, remove the syringe, then replace the buret handle. Then click the **OK**.

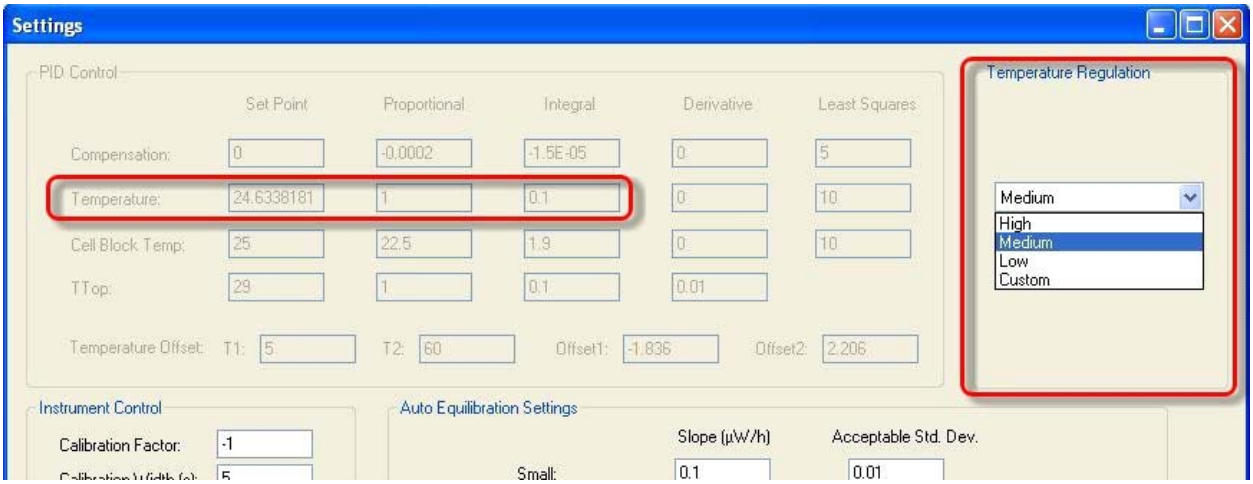


After the buret drive stops moving, it will be located at the top position and the buret position will be reported as 100% on the top of the NanoITC screen.

Appendix C: Temperature Regulation Control

The temperature regulation of the room environment can potentially have an impact on the baseline noise of the Nano ITC. Ideally the room temperature will be regulated within approximately 1° Celsius, with only gradual changes occurring. The instrument should also not be placed in an area that can receive direct sunlight, due to the sudden changes in the laboratory temperature that can occur on partially cloudy days.

It is not always possible to achieve the desired control over the ambient conditions, so a user-selectable setting has been provided in ITCRun software which can help to minimize the effects of the variation. A limited range of adjustment has been provided over one of the critical temperature control zones. There are three settings available to the user, labeled **High**, **Medium**, and **Low**. The selection control is provided in a drop-down menu that can be found in the Settings dialog box.



The following guidelines will help users make an appropriate selection. Make changes if there is a baseline stability issue, and use whichever setting gives the best low-noise and low-drift baseline characteristics.

High

Use this setting typically if the room temperature might experience temperature fluctuations that are wide ranging. The control is stronger at this setting and under some circumstances this may result in a small oscillation with a period of less than 10 seconds in the zone called **Temperature**. Use the System page to view the control band for the Temperature zone. If the peak-to-peak total variation in **Temperature** exceeds 200 micro degrees Celsius (0.0002°) then use a lower setting.

Medium

Medium is the default setting and it is what should generally be used unless there is a specific issue with the baseline.

Low

The Low setting may be useful in conditions where the room temperature changes are erratic rather than regular and cyclic. The temperature control is less strong in this setting and one of the stronger settings should be used instead if baseline drift occurs.

Determining the Best Setting

- Set up the instrument with degassed water or buffer in the Sample and Reference cells as well as the syringe. Set the stirring to 350 rpm for instruments with gold cells. Use 200 rpm for instruments with Hastelloy cells. Turn stirring on and allow the baseline to stabilize.
- Observe the baseline in the Monitor window for at least 10 minutes and note the baseline characteristics of peak to peak noise band and drift.
- Go to the Settings dialog box and try each of the three settings in turn. Allow at least 10 minutes of observation time and note the quality of the baseline in the Monitor page, and also the control band for the **Temperature** channel in the System page.
- If a small rapid oscillation occurs in the Temperature control band you may need to use a lower level setting for the Temperature Regulation control. This might also appear in the Monitor page as a small oscillation in the baseline.
- If the baseline slowly drifts in the Monitor page you may need to use a stronger setting for the Temperature Regulation control.