GE Healthcare

MicroCal™ Auto-iTC$_{200}$ system

Operating Instructions

Original Instructions

Table of Contents

1 Introduction

Purpose of the Operating Instructions

The Operating Instructions provide you with the instructions needed to handle the MicroCal Auto-iTC$_{200}$ in a safe way.

Prerequisites

In order to operate the MicroCal Auto-iTC$_{200}$ safely and according to the intended purpose the following prerequisites must be met:

- You should have a general understanding of the use of a personal computer running Microsoft™ Windows™ in the version provided with your product.
- You should be acquainted with the use of general laboratory equipment and with handling of biological materials.
- You must read the Safety Instructions in *Chapter 2* of these Operating Instructions.
- The system should be installed according to the instructions in *Chapter 3* of these Operating Instructions.
- You should understand the concepts of titration calorimetry.
- You must read and understand these Operating Instructions.

In this chapter

This chapter contains important user information and a general description of the MicroCal Auto-iTC$_{200}$ and its intended use.

1.1 Important user information

Read this before using the MicroCal Auto-iTC$_{200}$

All users must read the Safety Instructions in *Chapter 2* of these Operating Instructions before installing, using or maintaining the system.

Do not operate the MicroCal Auto-iTC$_{200}$ in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

The MicroCal Auto-iTC$_{200}$ is an Isothermal Titration Calorimeter system designed for bio-molecular interaction studies in research applications.

The MicroCal Auto-iTC$_{200}$ is intended for research use only and shall not be used in any clinical procedures or for diagnostic purposes.

Safety notices

These Operating Instructions contain WARNINGS, CAUTIONS and NOTICES concerning the use of the product, with meanings as defined below.

WARNING
WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION
CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

NOTICE
NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

Note: *A Note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A Tip contains useful information that can improve or optimize your procedures.*

Typographical conventions

Software texts and commands are identified by ***bold italic*** text. A colon is used to separate menu levels (e.g. ***File:Open*** refers to the ***Open*** option in the ***File*** menu).

1.2 Regulatory information

This section lists the directives and standards that are fulfilled by the MicroCal Auto-iTC$_{200}$.

Manufacturing information

Requirement	Content
Name and address of manufacturer	GE Healthcare MicroCal Products Group 22 Industrial Drive East, Northampton, Massachusetts, 01060 USA
Place and date of declaration	Northampton, Massachusetts, USA, Jan. 2010
Identity of person authorized to sign Declaration of Conformity	See EC Declaration of Conformity
Date of manufacture and serial number	The serial number contains the code for the year of the manufacture of the instrument. (the serial number takes the form of) xx.yy.zzz where yy = year of manufacture.

CE Conformity

Directive	Title
2006/42/EC	Machinery Directive (MD)
2006/95/EC	Low Voltage Directive (LVD)

Directive	Title
2004/108/EC	ElectroMagnetic Compatibility (EMC) Directive

International standards

Standard	Description	Notes
EN 61010-1, IEC 61010-1, CAN/CSA-C22.2 no. 61010-1	Safety requirements for electrical equipment for measurement, control and laboratory use	
EN 61326-1 (CISPR Group 1, Class A)	EMC emissions and immunity requirements for measurement, control and laboratory use	Harmonized with 2004/108/EC
EN-ISO 12100-1, 12100-2	Safety of machinery – Basic concepts, general principles and design	Harmonized with 2006/42/EC
EN-ISO 14121-1, 14121-2	Safety of machinery – Principles of risk assessment	Harmonized with 2006/42/EC

CE marking

CE

The CE marking and the corresponding Declaration of Conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked instruments, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation or explicitly authorized by GE Healthcare.

Regulatory compliance of connected equipment

Any equipment connected to the MicroCal Auto-iTC$_{200}$ should meet the safety requirements of EN 61010-1/IEC61010-1 or relevant harmonized standards. Within the European Union, connected equipment must be CE-marked.

Instrument safety compliance specifications

GE Healthcare MicroCal Auto-iTC$_{200}$ calorimeters carry the CUE Safety Certification Mark, authorized by TÜV America, a division of TÜV Süddeutschland, to signify that:

1 The instrument has been tested by an accredited Certification Body and meets applicable Canadian electrical safety standards/requirements (CSA/SCC).

2 The instrument has been tested by an NRTL (Nationally Recognized Testing Laboratory) and meets applicable United States electrical safety standards/requirements (ANSI/UL).

The instrument has been tested by a Competent and Notified Body for applicable EU Directives and meets applicable safety standards/requirements (EN/IEC).

1.3 Instrument

The iTC_{200} (Isothermal Titration Calorimeter, 200 µL cell) unit directly measures heat evolved or absorbed in liquid samples as a result of mixing precise amounts of reactants. A spinning syringe is utilized for injecting and mixing of reactants. Spin rates are user selectable; the usual range is 500 to 1500 rpm. The normal temperature operating range is 2°C to 80°C. Wetted cell surfaces are Hastelloy, which are resistant to most solutions; however, strong acids must be avoided.

Sample and reference cells are accessible for filling and cleaning through the top of the unit. The sample cell is on the left as one faces the front of the unit. A pair of identical coin shaped cells is enclosed within two shields; the inner shield is referred to as the jacket. Access stems extend from the top exterior of the instrument to the cells. Both the coin shaped cells and the access stems are completely filled with liquid during operation. This requires approximately 280 µL per cell even though the working volume of the cell is only 200 µL. The autosampler requires at least 400 µL per well in a 96-well plate or 350 µL per run in a centrifuge tube to be certain of filling the cell properly.

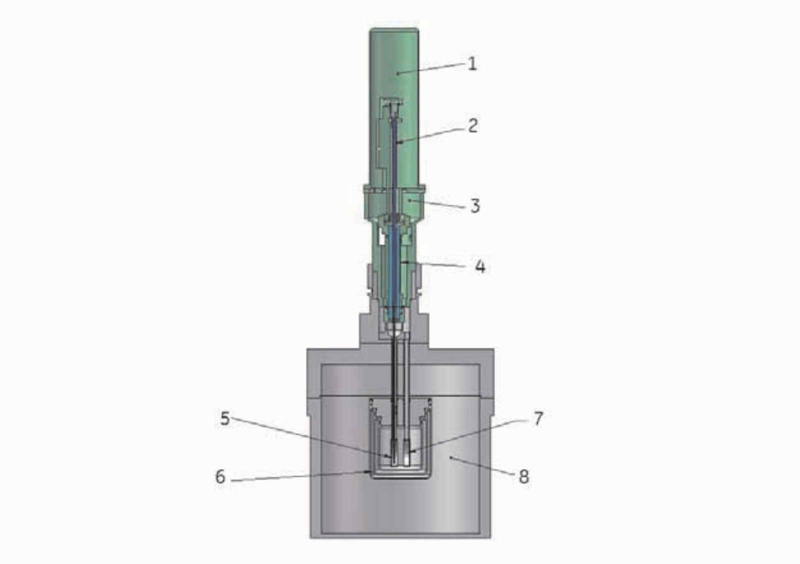


Figure 1-1. Principle drawing of ITC.

Part	Description	Part	Description
1	Pipette	5	Sample cell (with syringe)
2	Plunger screw (dark blue)	6	Adiabatic jackets
3	Stirring motor	7	Reference cell
4	Syringe (light blue)	8	Outer shield

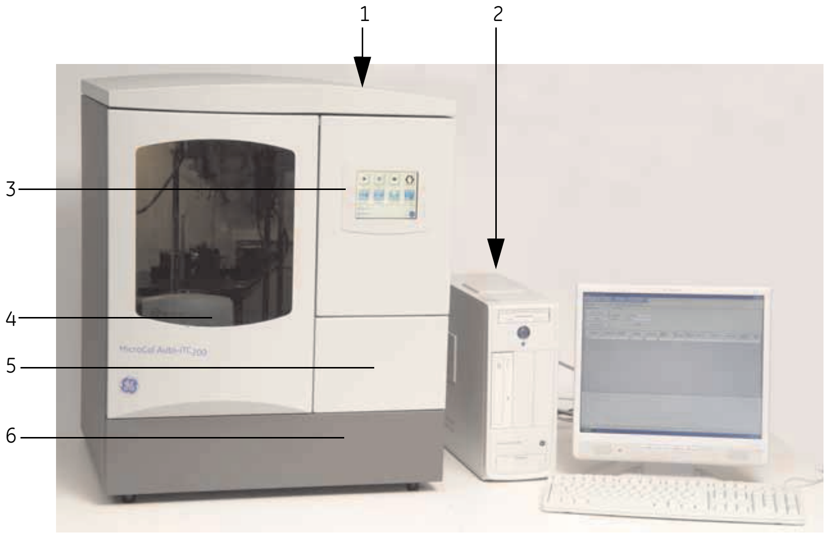


Figure 1-2. MicroCal Auto-iTC$_{200}$.

Part	Description	Part	Description
1	MicroCal Auto-iTC$_{200}$	4	iTC$_{200}$, inside
2	Controller	5	Drawer (contains (5) 30 ml tubes)
3	Touchscreen	6	Tray (Contains (4) 96 plate wells)

Temperature differences between the reference cell and the sample cell are measured, calibrated to power units and displayed to the user as well as saved to disk. The data channel is referred to as the DP signal, or the differential power between the reference cell and the sample cell. This signal is sometimes thought of as the "feedback" power used to maintain temperature equilibrium. Calibration of this signal is obtained electrically by administering a known quantity of power through a resistive heater element located on the cell.

In a typical experiment, the syringe containing a ligand is titrated (injected) into the cell containing a solution of macromolecule. An injection which results in the evolution of heat (exothermic) within the sample cell causes a negative change in the DP power, since the heat evolved chemically provides heat that the DP feedback is no longer required to provide.

The opposite is true for endothermic reactions. Since the DP has units of power, the time integral of the peak yields a measurement of thermal energy, ΔH. This heat is released or absorbed in direct proportion to the amount of binding that occurs. When the macromolecule in the cell becomes saturated with added ligand, the heat signal diminishes until only the background heat of dilution is observed.

With the MicroCal Auto-iTC$_{200}$ the entire experiment takes place under computer control. The user inputs the experimental parameters (temperature, number of injections, injection volumes) and the computer carries out the experiment. The resulting data is automatically analyzed, using fitting models to calculate reaction stoichiometry (n), binding constant (KD), enthalpy (H) and entropy (S), and displayed in the software and saved in an Excel™ spreadsheet. Origin® software, from OriginLabs, may also be used for more detailed analysis.

1.4 Control software

In order for the system to initialize properly, all components must be powered up in the correct order. First, boot up the computer and log in to Windows. Once Windows has started, power on the MicroCal Auto-iTC$_{200}$ by operating the switch at the rear of the unit. After several seconds, open the MicroCal Auto-iTC$_{200}$ software. This software controls the function of the ITC itself. If the option is selected, a real-time copy of Origin will open automatically, as well as the MicroCal Auto-iTC$_{200}$ control software. This copy of Origin is reserved for real-time data display, so for analysis, the user should open a separate copy.

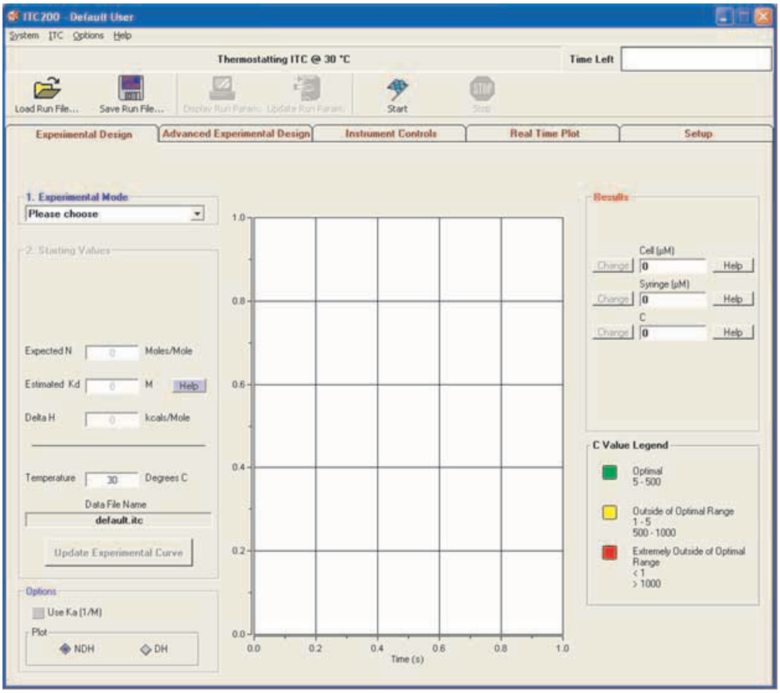


Figure 1-3. Instrument control software.

At startup, the line just below the menus will read ***System Initiation - Please Wait***, which is the current status of the instrument. After a few seconds, the system will begin heating or cooling to the preset temperature. Once the software has initialized, open the MicroCal Auto-iTC$_{200}$ software; this controls the automation and communicates with the iTC$_{200}$ software to perform experiments.

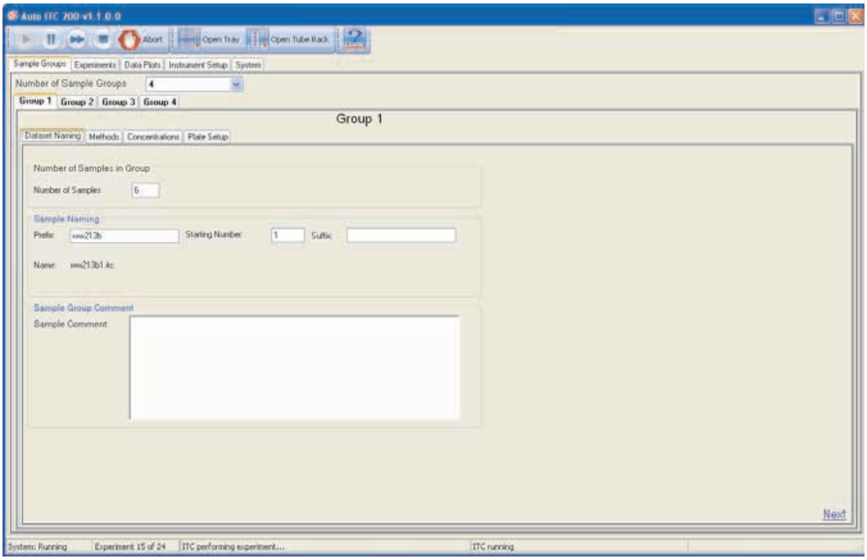


Figure 1-4. MicroCal Auto-iTC$_{200}$ control software.

The automation will start by initializing each component; various motors will move briefly.

2 Safety instructions

The points below are intended to enhance your safety awareness and to draw your attention to risks which only you, the operator, can prevent. While GE Healthcare works to ensure that the instrument is designed and tested to be as safe as possible, proper handling is also critical. The operators should be responsible people trained in basic laboratory protocol, and they should be familiar with the possible hazards before operating this instrument. All instrument modifications should be performed only by personnel trained by GE Healthcare. Equipment damage, personal injury or even death may result if this equipment is operated, altered or maintained by untrained personnel or in an irresponsible or improper manner.

2.1 Safety precautions

Introduction

Before installing, operating or maintaining the system, you must be aware of the hazards described in the user documentation. Follow the instructions provided to avoid personal injury or damage to the equipment.

The safety precautions in this section are grouped into the following categories:

- General precautions
- Flammable liquids
- Personal protection
- Installing and moving the instrument
- System operation
- Maintenance

General precautions

WARNING!
Provide proper electrical power to the instrument. This should be 100 – 240 Volt, 50/60 Hertz alternating current, with a Ground Fault Circuit Interrupter (GFCI). Some power strips, such as the one provided by GE Healthcare with your instrument, contain a GFCI. All power plugs and cords should be 3-prong, grounded cables or outlets.

WARNING!
In case of fire, unplug instrument.

WARNING!
Do not operate the MicroCal Auto-iTC$_{200}$ in any other way than described in the MicroCal Auto-iTC$_{200}$ system and/or iTC$_{200}$ manuals.

WARNING!
Make sure the rear power connector is always accessible.

WARNING!
Use caution when using solutions near the instrument. If any liquid is spilled on or around the instrument, unplug the instrument immediately and wipe it up. If there is any possibility that liquid may have leaked into the instrument case, contact GE Healthcare immediately. Do not plug the instrument into any electrical outlet until the problem is resolved.

WARNING!
This instrument is not designed to the Medical Devices Directive 93/42/EEC and should not be used for medical purposes and/or in the diagnosis of patients.

NOTICE
The iTC$_{200}$ cells are constructed out of Hastelloy. Strong acids must be avoided.

Using flammable liquids

WARNING!
A fume hood or similar ventilation system shall be installed when flammable or noxious substances are used.

WARNING!
Fire Hazard. Before starting the system make sure that there is no leakage.

Personal protection

WARNING!
Always use protective glasses and other personal protective equipment appropriate with the current application, to ensure personal safety during operation.

WARNING!
The operator should always follow proper laboratory procedures in handling and disposing of volatile or hazardous solutions. Please refer to MSDS requirements for chemical hazards and PPE equipment requirements.

WARNING!
This instrument is used for a wide variety of experiments that can utilize potentially hazardous materials. Use of these could cause exposure to biological, chemical and radiation hazards depending on the user's experiments. Users should educate themselves about the samples they are using to avoid these hazards.

Installing and moving the instrument

WARNING!
Power cord. Only use power cords delivered or approved by GE Healthcare.

WARNING!
Do not block the ventilation inlets or outlets on the system.

WARNING!
Installing the controller. The controller should be installed and used according to the instructions provided by the documentation included in the shipment.

WARNING!
Replace fuses ONLY with the same type and rating as the spare fuses that are provided with the original shipment.

WARNING!
Access to power switch and power cord. Do not block the rear and side panel of the instrument. The Power switch must always be easy to access. The power cord must always be easy to disconnect.

NOTICE
Disconnect power. To prevent equipment damage, always disconnect power from the MicroCal Auto-iTC$_{200}$ before an instrument module is removed or installed or a cable is connected or disconnected.

CAUTION
The MicroCal Auto-iTC$_{200}$ without its special crate weighs approximately 90 kg (200 lbs). A suitable lifting device or at least four people are required to lift the instrument.

System operation

WARNING!
All solutions in the cells must be cooled down below 40°C before removal. Any higher temperature may cause the syringe to break, and will increase the dangers of most hazardous solutions.

WARNING!
Do not place vessels containing liquid on top of the instrument or inside the cabinet. Spilled liquid is an electrical hazard.

CAUTION
Waste tubes and containers shall be secured and sealed to prevent accidental spillage.

NOTICE
Never allow liquid in the cells to freeze. The expansion of the liquid can distort the cells and rupture the most critical sensor, causing irreparable damage.

NOTICE
The MicroCal Auto-iTC$_{200}$ should always be moved in its normal operating orientation. Other orientations will subject delicate sensors inside the instrument to stress.

Maintenance

WARNING!
Replace fuses ONLY with same type fuses. Several spare fuses are provided with the original shipment and the power receptacle is labeled with the correct type.

WARNING!
Repairs, alterations or modifications other than those described in this manual must only be carried out by a GE Healthcare specialist, or with explicit directions from a GE Healthcare technician. Removal or modification of any cover or component could result in an unsafe or easily damaged instrument. The GE Healthcare service department will be happy to answer any questions and provide parts and service when necessary.

WARNING!
Only spare parts that are approved or supplied by GE Healthcare may be used for maintaining or servicing the system.

WARNING!
Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.

WARNING!
Hazardous chemicals during run. When using hazardous chemicals, flush the entire system tubing with distilled water, before service and maintenance.

WARNING!
Hazardous chemicals during maintenance. When using hazardous chemicals for cleaning, wash the system with a neutral solution in the last phase or step.

WARNING!
Decontaminate the equipment before decommissioning to ensure the removal of all hazardous residues.

WARNING!
Contrad 70 (Decon 90) is corrosive and therefore dangerous to health. When using hazardous chemicals, avoid spillage and wear protective glasses, gloves, and other suitable personal protective equipment.

WARNING!
This instrument is designed with interlocks to prevent operation when the door is open. If these interlocks are disabled and/or any of the cabinet's panels are removed there is a potential for pinch, puncture and electrical injury. Only trained service personnel should service this instrument under these circumstances.

2.2 Labels

Labels on the instrument

The illustration below shows an example of the identification labels attached to the rear of the iTC$_{200}$ instrument.

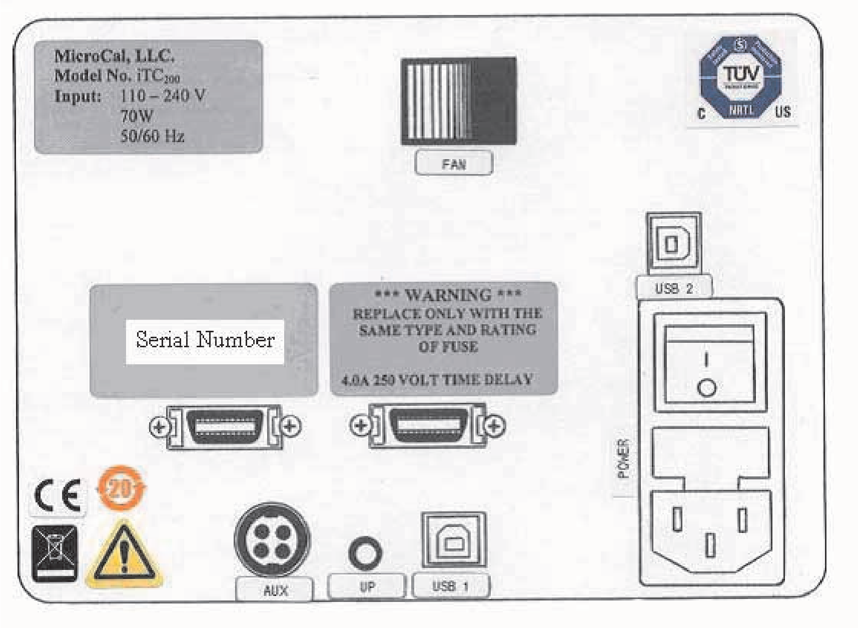


Figure 2-1. Back panel of the iTC$_{200}$ instrument.

Figure 2-2. Rear panel of MicroCal Auto-iTC$_{200}$ system.

Symbols used in safety labels

	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	**Warning!** Read the user manual before using the system. Do not open any covers or replace parts unless specifically stated in the user manual.
	The system complies with applicable European directives.

Labels concerning hazardous substances

	This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.
	This symbol indicates that the product contains hazardous materials in excess of the limits established by the Chinese standard SJ/T11363-2006. Requirements for Concentration Limits for certain Hazardous Substances in Electronics.

Emergency procedures

In an emergency situation, do as follows to stop the run:

Step	Action
1	Disconnect the equipment from the power outlet.

Power failure

MicroCal Auto-iTC$_{200}$

- The run is interrupted immediately, in an undefined state.

- The data collected up to the time of the power failure is saved.

Controller

- The controller shuts down, in an undefined state.
- The MicroCal Auto-iTC$_{200}$ run is interrupted immediately, in an undefined state.

2.3 Recycling procedures

The equipment shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.

Disposal, general instructions

When taking the MicroCal Auto-iTC$_{200}$ out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Recycling of hazardous substances

The MicroCal Auto-iTC$_{200}$ contains hazardous substances. Detailed information is available from your GE Healthcare representative.

Disposal of electrical components

Waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of GE Healthcare for information concerning the decommissioning of equipment.

3 Installation

NOTICE
The MicroCal Auto-iTC_{200} must be installed by trained GE Healthcare personnel.

This section provides information about the installation of MicroCal Auto-iTC_{200} .

Figure 3-1. MicroCal Auto-iTC_{200} with controller.

Any equipment connected to the MicroCal Auto-iTC_{200} must fulfill applicable standards and local regulations.

3.1 Site requirements

The MicroCal Auto-iTC_{200} with Controller requires about 1.2 meters of normal bench space (ca. 70 cm wide). This location should be away from strong drafts, room temperature fluctuations, intense sunlight, vibrations and strong electrical or magnetic fields (as may be produced by an NMR, microwave oven, large motors or refrigeration units). In addition, the mains power source (100 to 240 VAC) should be properly grounded and free from voltage fluctuations, harmonic distortions, power dips and spikes. The AC power line should be dedicated to the MicroCal Auto-iTC_{200} and should not share that power with additional equipment.

Although the power filtering in the MicroCal Auto-iTC$_{200}$ instrument is adequate for most laboratory environments, some disturbances may affect the performance of the instrument and it may be necessary to have the AC Mains power source evaluated (see table below) or install a power conditioner. Since power source problems can be manifested in many different ways, it is not possible to recommend a power conditioner for all situations. It is recommended that you test a power conditioner, at your location, before you purchase it. If you believe you are experiencing power source related problems, please contact a GE Healthcare field engineer.

Table 3-1. Power supply requirements.

AC Mains Requirements	
Specification	**Requirement**
Voltage Regulation	100 to 240 VAC
Frequency	50/60 Hz
Power	300 Watts
Fuses	MicroCal iTC200: (2) 4.0A, 250V, Time Delay; MicroCal Auto-iTC200: (2) 5.0A, 250V, Fast Acting
Protective Earth Terminals	Internal/external marked

It is emphasized that room temperature fluctuations due to the cycling on/off of heating and cooling systems, strong air currents, sunlight directly on the instrument and through space electromagnetic waves may cause subtle performance problems.

Table 3-2. Environmental operating requirements.

Environmental (Operating) requirements	
Temperature	10 to 28°C
Humidity	≤ 70% RH
Atmospheric Pressure	Normal

Table 3-3. Autosampler fluid requirements

Fluid	Requirement
Nitrogen	≥ 20 and < 30 psi (pressure regulated) laboratory nitrogen or other compressed inert gas
Water	Distilled
Methanol	≥ 99% pure ("HPLC Grade" is recommended)

Fluid	Requirement
Contrad® 70 also known as Decon 90 (detergent)	20% concentration in water

3.2 Transport

CAUTION
The MicroCal Auto-iTC$_{200}$ instrument weighs approximately 135 kg (300 lbs) when it is delivered in a black plastic shipping crate. All movement of the crate before and during installation is the sole responsibility of GE Healthcare personnel.

CAUTION
The MicroCal Auto-iTC$_{200}$ instrument without its special crate weighs approximately 90 kg (200 lbs). Special lifting equipment or at least four people are required to lift the instrument.

Before moving the system:

- Disconnect all cables and tubing connected to peripheral components and liquid containers.
- Remove all items from the top of the system.
- Grasp the system under the two sides.

3.3 Unpacking

NOTICE
The MicroCal Auto-iTC$_{200}$ instrument must be unpacked and installed by trained GE Healthcare personnel.

Document any damage and contact your local GE Healthcare representative.

3.4 Set up

Parts and accessories

The main part of the MicroCal Auto-iTC$_{200}$ is the large automated box. A controller is provided with the instrument and contains proprietary software and calibration constants specific to the automation and the iTC$_{200}$. A set of reagent and waste bottles is plumbed to the rear of the autosampler.

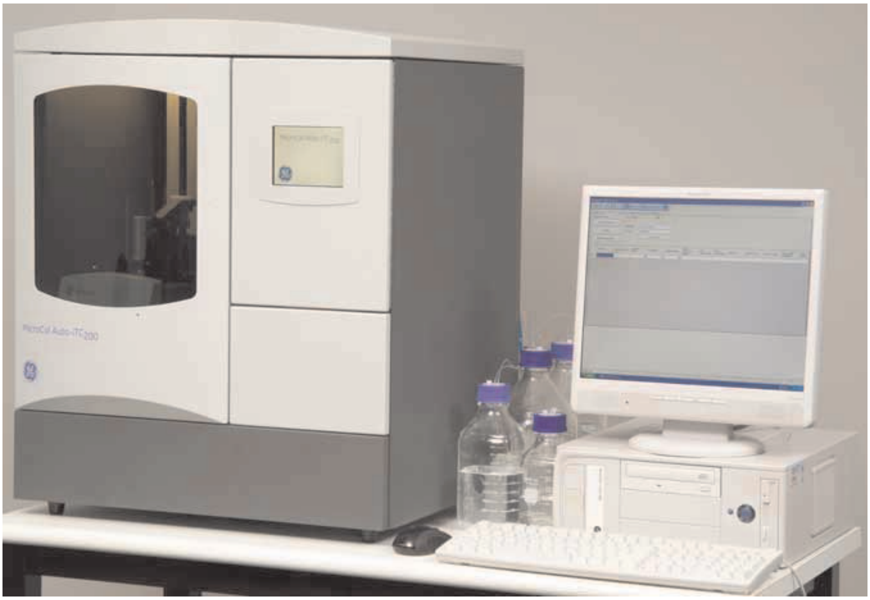


Figure 3-2. MicroCal Auto-iTC$_{200}$ running with controller.

The microcalorimeter itself sits inside the main portion of the automated box, in the lower left corner. Three robotic arms, one holding the titration pipette and two holding cannulas for fluid transfer, move within the box. A tube rack drawer at the right side of the box allows the user to insert samples in 30 mL centrifuge tubes. The 96-well plate tray at the bottom holds up to four plates of sample. The tray drawer and tube drawer both have potential to cause injury by pinching a body part between the face plate and the instrument's frame; users should make sure their hands are out of the drawers before closing them.

CAUTION
The tray drawer and tube drawer both have potential to cause injury by pinching a body part between the face plate and the instrument's frame.

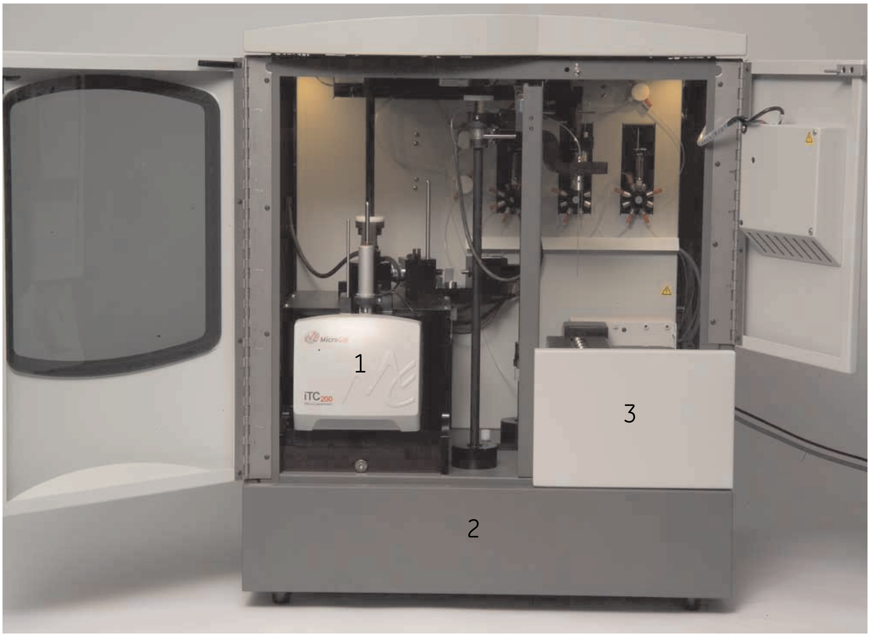
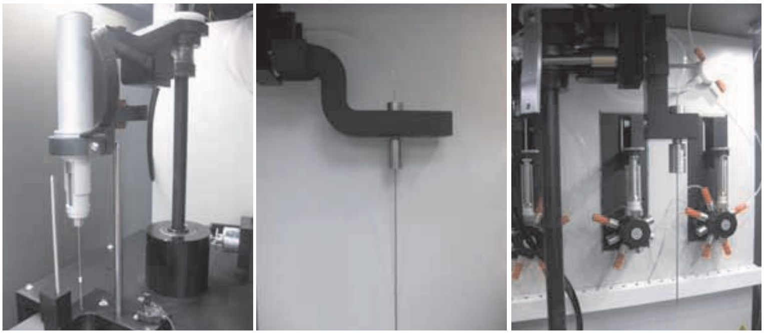


Figure 3-3. MicroCal Auto-iTC$_{200}$ internal view.

Part	Description
1	iTC$_{200}$
2	96 Well plate tray
3	Tube rack drawer



Pipette arm Cell arm Titrant transfer arm

Figure 3-4. MicroCal Auto-iTC$_{200}$ autosampler arms.

Connections on the lower left of the rear panel are provided for pressurized nitrogen, the USB data connection to the controller, power, and reagent and waste bottles.

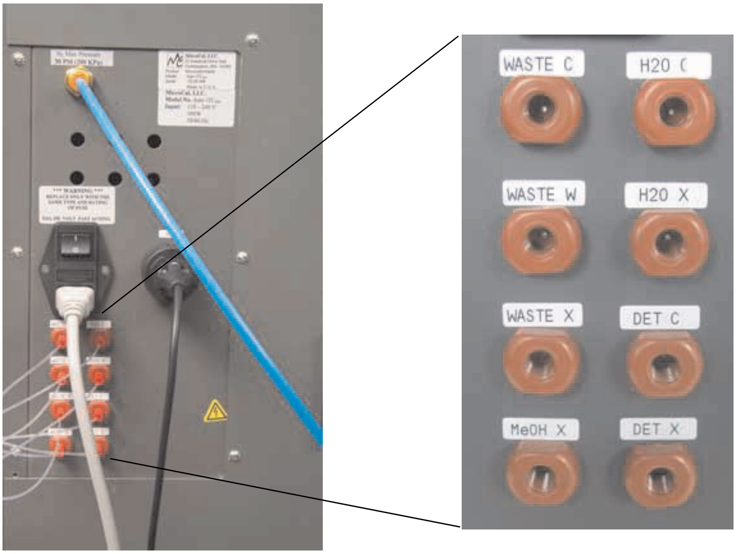


Figure 3-5. MicroCal Auto-iTC$_{200}$ connections.

Nitrogen supply

The pressure relief valve shipped with the instrument must be connected between the pressure regulator from the nitrogen supply and the nitrogen feed line to the MicroCal Auto-iTC$_{200}$. This is highly recommended to prevent injury to personnel or damage to the instrument.

Reagents

The autosampler requires distilled water, ≥ 99% pure methanol ("HPLC Grade" is recommended), and whatever detergents or cleaners may be desired. Currently the system is set up to use Contrad 70 (Decon 90), diluted to 20%, as the detergent. Contrad 70 is manufactured by Decon Laboratories and contains dodecylbenzensulfonic acid, potassium hydroxide, sodium citrate and sodium laurel ether sulfate. It is biodegradable and easily rinsed. Bottles and tubing are provided with the instrument.

> **WARNING!**
> Methanol is highly volatile and can be hazardous to humans.

Storage containers should be kept tightly closed. When transferring it, the user should be in a well-ventilated area with no ignition sources and wearing gloves. Methanol can be absorbed through the skin. Do not allow methanol to be swallowed or to come in contact with skin or eyes. If accidental exposure occurs, flush the affected area with water. If methanol is swallowed, or there is significant skin or eye exposure, seek medical help.

Sample containers

The autosampler takes sample in 96-well plates and 30 mL centrifuge tubes. Replacements may be purchased through GE Healthcare, or directly from a supplier.

NOTICE
Using supplies other than ones recommended here may lead to improper performance and potentially autosampler damage, and may void the warranty.

Centrifuge tubes 96-Well Plates Well Plate Covers

Figure 3-6. Sample containers.

3.5 Validation

After installation it is recommended that a titration of a known system be performed to test that the instrument has been installed correctly.

3.6 Configuring MicroCal Auto-iTC$_{200}$ controller for networking

On a MicroCal Auto-iTC$_{200}$ controller, there are several actions that must be performed for the networking and MicroCal Auto-iTC$_{200}$ control software to work properly. First, the InitDT watchdog program must be removed, and once the computer has been configured for the local domain, two folders must be given special permissions.

Uninstallation:

1 Click on the ***START*** button on the lower left side of the screen, and then on ***Control Panel***.

2 In the ***Control Panel*** window, click on ***Add or Remove Programs***.

3 Find ***InitDTSetup*** in the program list. Click the ***Remove*** button by it. In the next window, click ***Yes*** to confirm the removal.

Special Permissions:

The iTC$_{200}$ software and Origin both need to be able to write data into their own folders. In order to allow a non-administrator to use this software, read/write privileges must be set for all users for the iTC$_{200}$ and Origin folders, including all subfolders.

The folder properties must allow Read and Execute, List Folder Contents, Read, and Write, as shown below.

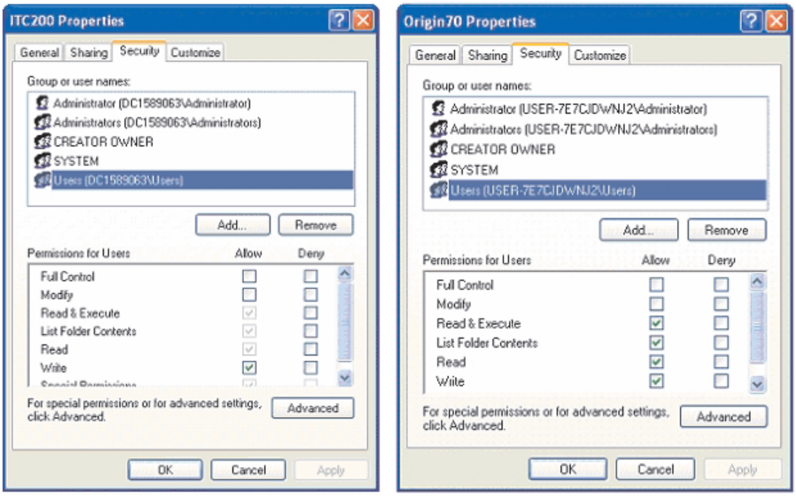


Figure 3-7. Configuration dialogs.

4 Operation

4.1 Procedure before a run

Note: *See Section 1.4 for control software information.*

On / Off Instructions

Turning the MicroCal Auto-iTC$_{200}$ cell on

Once the MicroCal Auto-iTC$_{200}$ cell has been cabled to the PC, it is ready to use. At the rear of the cell unit is a power on/off switch, which functions as the master power switch and must be in the "on" position. It can be turned to "off" when the MicroCal Auto-iTC$_{200}$ cell will not be used for long periods of time i.e., weekends, holidays, etc.

NOTICE
The user interface programs, iTC$_{200}$ and MicroCal Auto-iTC$_{200}$ have to be running for the cell and autosampler to function properly even though the power switch is in the "on" position.

Leaving the power on

During frequent "on" periods, the master power may be left as long as the user interface program, iTC$_{200}$, is running. The software automatically ensures that the system does not incur any damage and keeps the iTC$_{200}$ cell ready.

Periods of inactivity

GE Healthcare recommends that the iTC$_{200}$ and the MicroCal Auto-iTC$_{200}$ application be closed and the master power be turned off, when the system will not be used for extended periods of time.

4.2 Basics of performing a run

In order to perform a basic ITC titration experiment, the user must load the samples for the cell and syringe into the autosampler, set up the run parameters, validate the parameters, and click ***Start***. The ***Groups*** and ***Experiments*** tabs of the automation software provide guidelines for setting up the run (see sections 4.4 and 4.5 for a more complete description). Within the ***Groups*** tab, enter the number of titration runs, the ITC and automation methods (for a first run, WATER.inj and Plates Standard.setup are recommended), and the concentrations, and select the ***Plate Setup*** subtab. A color-coded image of the sample tray and tube rack will appear. Load the proper number of 96-well plate wells with 400 μL of sample for the cell and 120 μL for the syringe, or load the 96-well plate with the syringe sample, and load a 30 mL centrifuge tube with at least 350 µL per run of the cell sample.

The easiest way to see if the plate is loaded properly is to hold it up to an overhead light. The example plate below is loaded with alternating 400 µL for the cell and 120 µL for the syringe.

Figure 4-1. filled 96-well plate.

Cover the tray with a Zone Free cover (Excel Scientific EZ-Pierce Zone-Free Microplate Film), being careful that the holes in the glue are centered over the holes in the plate itself.

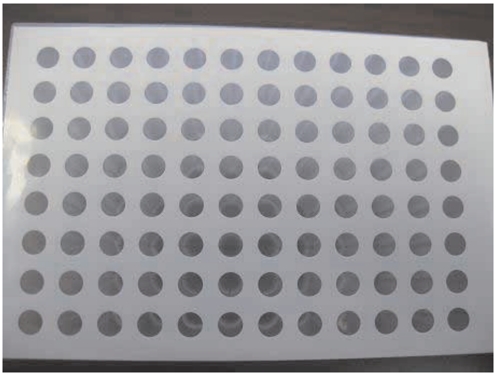


Figure 4-2. 96-well plate with cover.

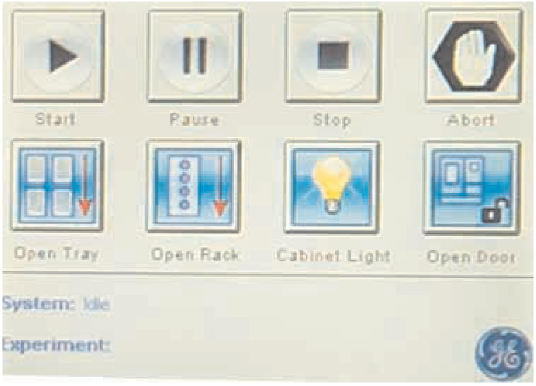


Figure 4-3. Touchscreen layout.

Use the buttons on the touchscreen to open and close the tray and rack drawer, and load the plates and/or tubes into the autosampler. When loading the 96-well plate, slide the edge of the plate under the lip down the center of the tray, and make sure the outer edge of the plate snaps down into position (see *Figure 4-4*). Once in, the plate should be secure. To remove the plate, pull up gently on the outer edge to release the snaps, slide it toward the outside edge to clear the lip, and lift up. When loading the centrifuge tube, remove the lid over the tube rack, slide the centrifuge tube into the cavity, and replace the lid. The lid opening mechanism must be at the rear. Make sure the lid is all the way in position before using the touchscreen or software to close the drawer again.

Figure 4-4. 96-well plate tray.

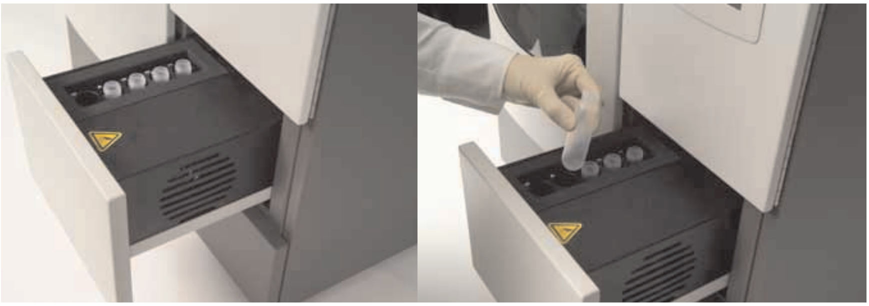


Figure 4-5. Tube rack drawer.

Switch the MicroCal Auto-iTC$_{200}$ control software to the ***Experiments*** tab and click ***Import Sample Groups*** to load that information into this spreadsheet. Click the ***Validate*** button. A window will pop up, giving total reagent consumption. Make sure enough of each reagent is available. Click the **Start** arrow button in the main button panel or on the touchscreen. The autosampler will home and start the pre-run cleaning routine. It will then load the cell and syringe and start a titration run. After each run, automated cleaning and reloading will be performed. After the last run, the autosampler will clean the cell, syringe, and internal tubing and mechanisms, and come to a stop.

Once a titration run starts, the real-time data will be displayed in the ***Data Plots*** tab, in the ***Real Time Display*** subtab. After each scan, an automatic data analysis will run and the results will appear in the ***Results Plot*** subtab, showing a scatter plot of the fitted stoichiometry (n), binding constant (K_D), and raw heat per injection (ΔH), as well as the calculated values for the Gibbs free energy (ΔG) and entropy (ΔS). In addition, clicking the ***Show Data*** button in the ***Experiments*** tab will bring up an Excel spreadsheet of the same numbers, as well as graphs of the raw data and normalized ΔH curve that appear when the curser hovers over the n and K_D columns.

4.3 Main buttons

The main control buttons appear both on the upper left of the software window and on the touchscreen in the front of the instrument. ***Start*** will start an experiment that has been loaded and validated. ***Pause*** will cause the automation to pause where it is, allowing the user to make changes in the run parameters or check on syringes. ***Fast Forward*** will stop the current ITC run and reload and start the next run. ***Stop*** will stop the instrument in an orderly fashion; the current ITC run will complete, and the post-run cleaning will be performed, and then the instrument will return to the idle state. ***Abort*** will stop whatever automation is running; if the ITC is currently performing a run, it will complete that run but the post-run cleaning will not happen. The ***Open Tray*** and ***Open Tube Rack*** buttons open and close the two drawers. The ***About*** button gives software version information. Some users may have version 1.0.0.0 of the MicroCal Auto-iTC$_{200}$ control software. There are a few features depicted in version 1.1 that may not be available in version 1.0. See chart below.

Location	Function	v1.1.0.0	v1.0.0.0
Tool Bar	Fast Forward button	Available	Not available
Methods Tab	Save Sample check box	Available	Not available
	Subtraction Method drop down	Not available	Available
Experiments Tab	Export List to Spreadsheet button	Available	Not available
Real time display Tab	Time Left progress bar	Available	Not available
Results Tab	Data Displayed	Available	Fewer columns available (no Offset, ΔG, or ΔS)
Results Plot Tab	Individual results plot tab	Available	Not available

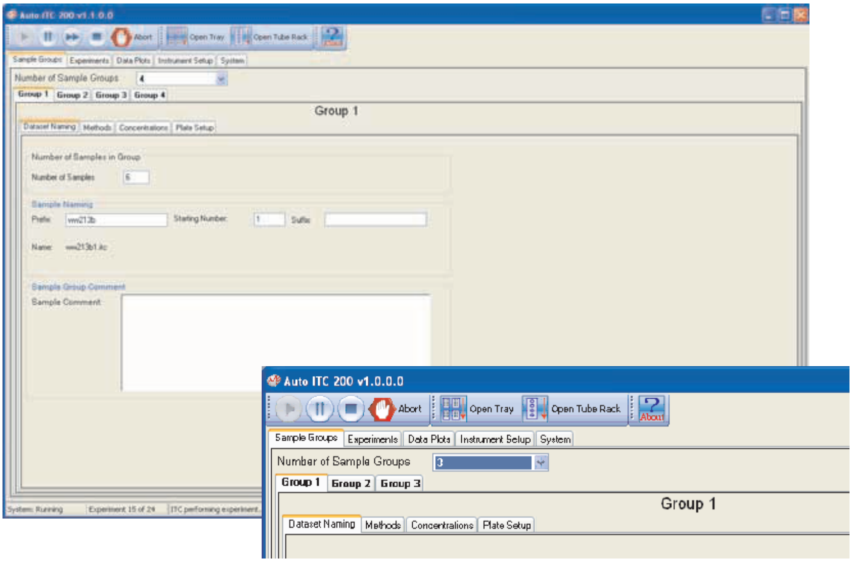


Figure 4-6. MicroCal Auto-iTC$_{200}$ control software showing version 1.0 as an inset.

4.4 Sample groups

This tab populates the spreadsheet that instructs the automation software what samples to run and where to collect them. The ***Number of Sample Groups*** dropdown box allows the user to choose from 1 to 10 groups. Each group has a tab; all the groups have the same subtabs. A group is a series of runs, all with the same run parameters. It will take samples sequentially from a plate or plates, and number the data files sequentially. This screen may also be used as a shortcut, entering whichever parameters are the same for a series of runs, and then editing the spreadsheet for the parameters that are not the same.

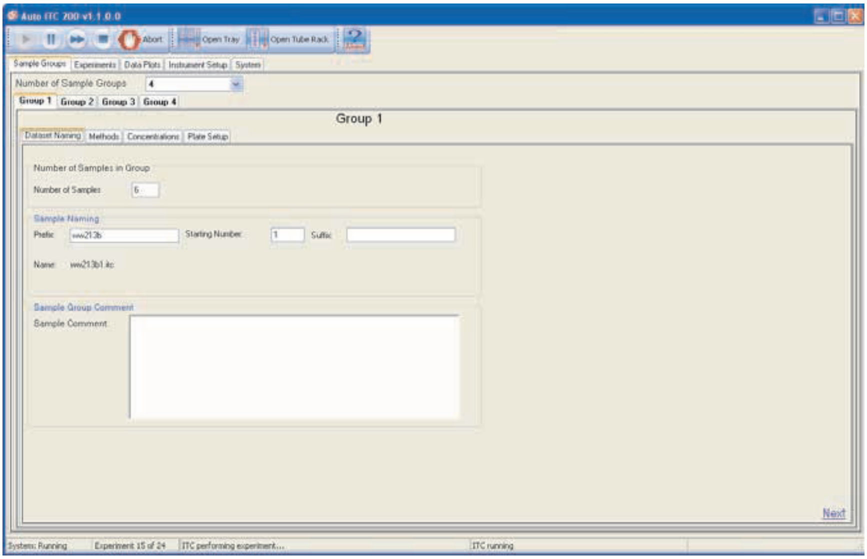


Figure 4-7. MicroCal Auto-iTC$_{200}$ control software.

Dataset naming

This subtab specifies the naming of the runs in the group. ***Number of Samples*** means the number of ITC runs in the group. The ***Sample Naming*** sections allows the user to specify the prefix, starting number, and suffix for the filenames. Each succeeding run will use the same prefix and suffix, and will increment the file number.

Methods

This subtab specifies the run methods for the series of experiments. The ***Automation Method*** dropdown allows the user to choose a method for the automated cleaning and loading. The ***Save Sample*** checkbox allows the user to have the reacted sample returned to the 96-well plate after the run has finished. The ***ITC Run Method*** allows the user to choose a previously saved *.inj iTC$_{200}$ runfile that contains the desired parameters for the ITC performance.

The ***Analysis Method*** dropdown allows the user to choose the post-run data analysis method they wish to use. The ***Load Cell from Tube?*** and ***Pre-Rinse?*** buttons indicate whether the selected method takes sample for the cell from the 96-well plate or the tube rack and whether or not the selected method includes a pre-rinse or sample saving routine.

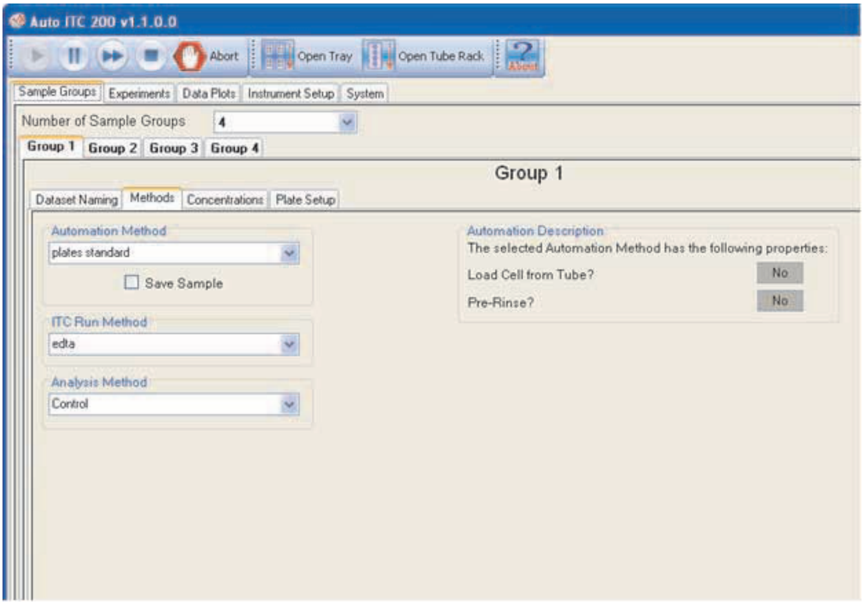


Figure 4-8. Methods tab.

Concentrations

This subtab allows the user to specify the concentrations for the syringe and cell for the group of runs. If these are not the same for all runs, the user will need to edit the spreadsheet to reflect the actual concentrations. These will be used for the automated post-run analysis, so incorrect concentrations may result in incorrect interpretation of the data.

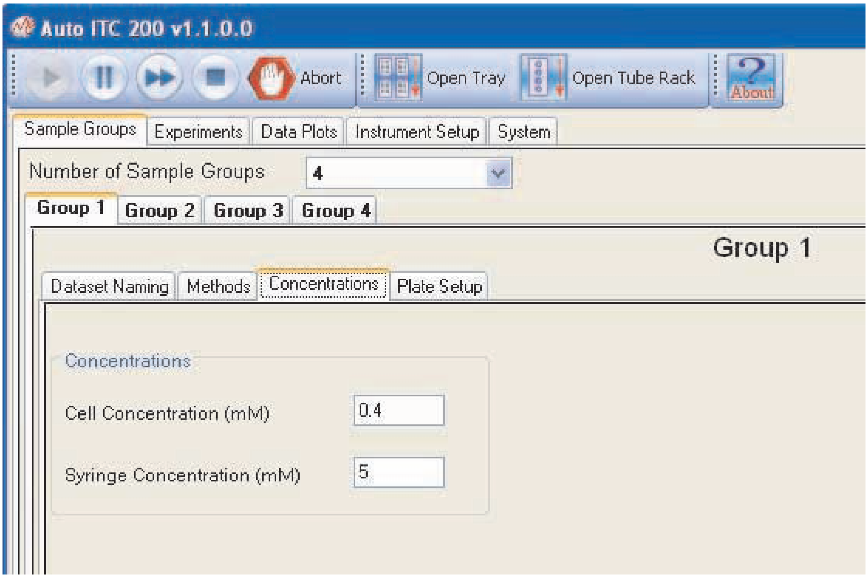


Figure 4-9. Concentrations tab.

Plate setup

This subtab allows the user to specify the source for the cell and pipette. Under the ***Starting Well*** section, the dropdown box gives a choice of the four trays, and the ***Well*** text box should contain the number of the well to use for the first experiment in the group. The well should be specified by row letter and column number, with no punctuation or spaces. The display to the right shows which wells are being used. For subsequent groups, the software will automatically designate the next wells; the user may override the automatic selection and choose a different starting well. Click on the ***Tube Rack*** tab below the plates to select the tube rack. Clicking a different tube will cause the automation to load the cell from that tube; only one tube may be used per group.

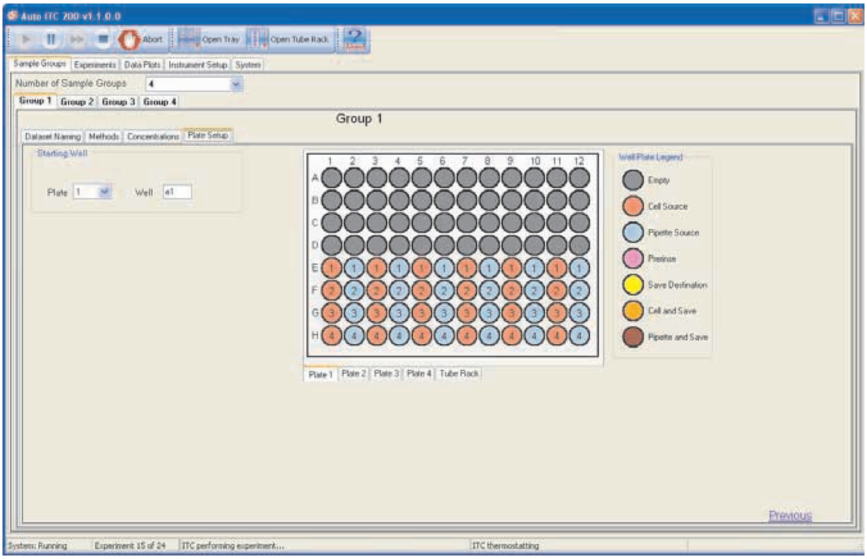


Figure 4-10. Plate setup tab.

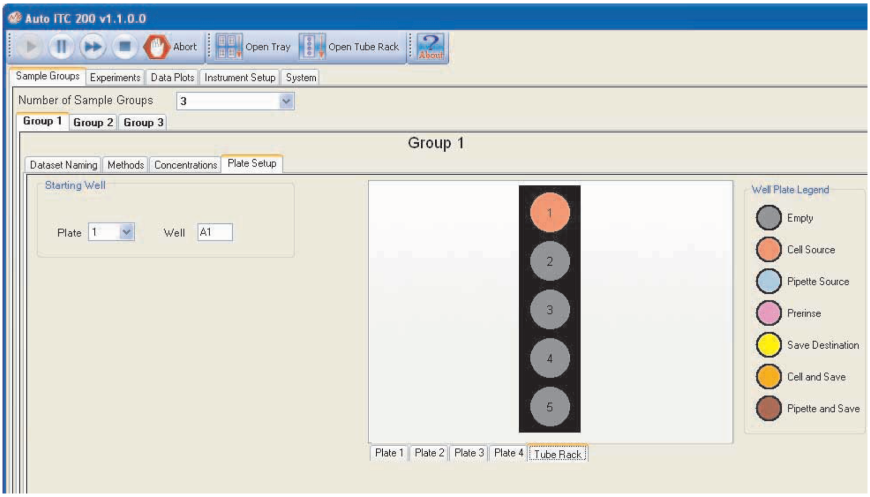


Figure 4-11. Plate setup tab; tube rack.

4.5 Experiments

This tab contains the details of each run programmed into the instrument, in a spreadsheet. The experimental run parameters may be loaded from a previously saved file using the ***Import Experiments*** button, or the user may enter the information into the ***Sample Groups*** tab and then click ***Import Sample Groups*** to load that information into this spreadsheet. Once here, the parameters may be edited. Click ***Validate*** to confirm the run parameters. A screen will pop up giving the amount of water and reagents required for the full series. Once a set of experiments has been validated, the ***Start*** button will become active.

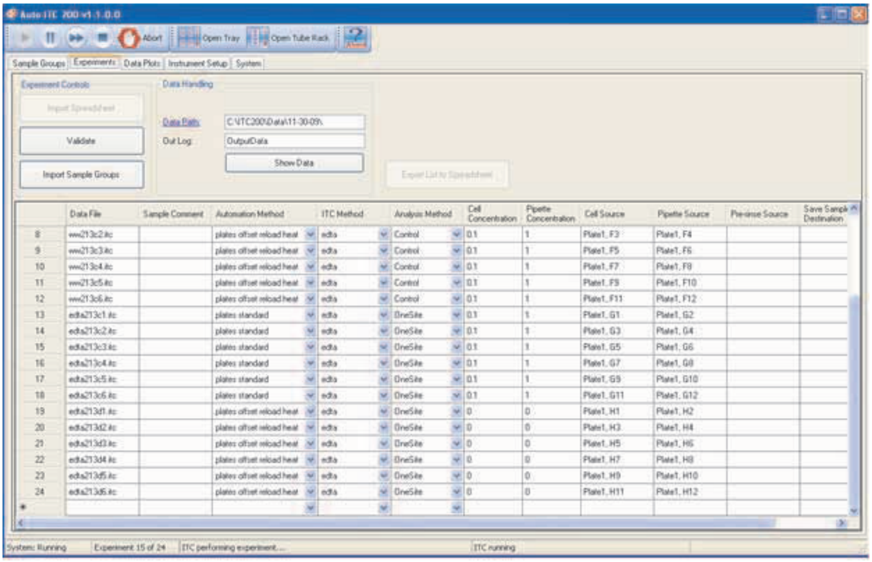


Figure 4-12. Experiments tab.

The ***Data path*** refers to the directory where the output data will be stored. The ***Output Results Log*** is the filename of the Excel spreadsheet where the automated data analysis will be stored. The ***Show Data*** button will open that Excel spreadsheet. The Excel output spreadsheet contains the run parameter information for each run and the automatically analyzed results. The ***Export List to Spreadsheet*** button is active while the system is not performing a run, and exports the displayed grid to an Excel file for later use.

The parameters that may be adjusted in this grid are the data file name; sample comment; automation and ITC methods; analysis method; cell and syringe concentrations; cell and pipette sources; and the pre-rinse source and saved sample destination.

The data file name is the name that will be given to the raw output file; for analysis in Origin it should not be more than 11 characters long. The sample comment will be saved as a comment in the output file, and is for reference only. The sample prep and ITC methods dropdowns allow the user to change the procedures that the automation and the iTC$_{200}$ will use to perform the run; each method must be previously saved under that filename as the appropriate file type.

The analysis method directs the post-run automated analysis, specifying the type of curve fitting. The cell and syringe concentrations should be entered in millimolar. The cell and pipette source columns give the well plate or tube rack location that the sample will be taken from, and must be in the following format: platenumber, rowcolumn.

The last two columns specify the source for the pre-rinse solution, if the method includes a pre-rinse, and the destination for the saved sample, if the method will save the sample.

4.6 Data plots

This tab displays the data being generated by the current set of titration runs. The ***Real Time Display*** subtab shows the raw DP signal from the current titration run. The ***Rescale*** and ***Baseline View*** buttons allow the user to view different parts of the trace. Clicking and dragging a section of the window will zoom in to the selected area. The time left until the end of the current titration run is also displayed.

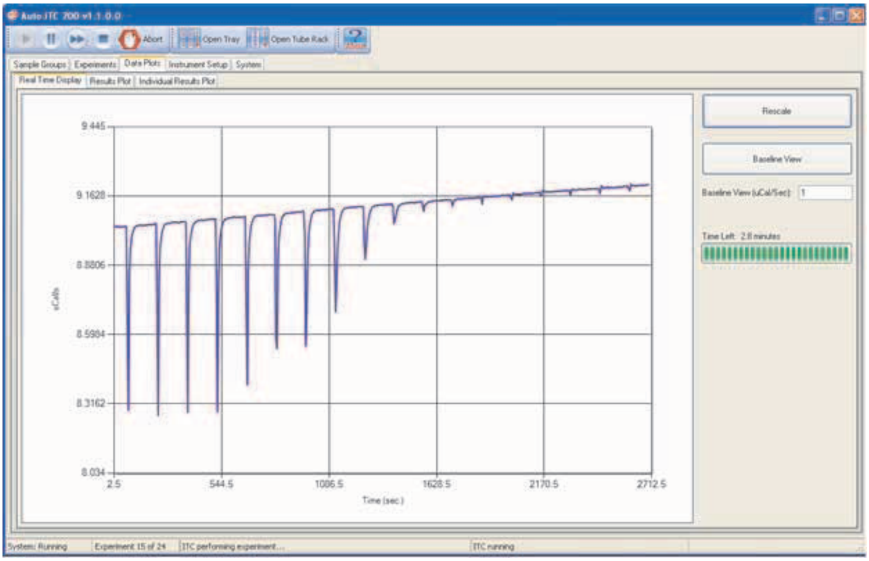


Figure 4-13. Real time display tab.

The ***Results Plot*** subtab shows the analyzed stoichiometry, K_D, ΔH, offset, Gibbs free energy, and entropy for all the completed runs in the set. The offset referred to here is the offset used during analysis to adjust the final injections heats to zero. The graph will show a scatter plot of whichever parameter is selected in the upper right of the screen, graphed over the number of each scan.

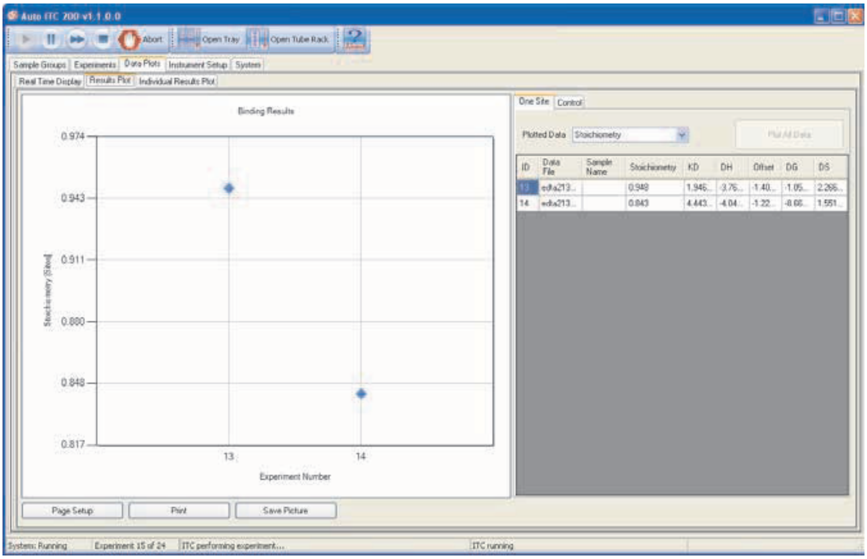


Figure 4-14. Results tab.

The ***Individual Results Plot*** subtab shows the analyzed data for each run. Any complete scan may be selected from the ***Plotted Data*** drop-down box. For each run, users may view the raw data, or the NDH or DH plotted for each injection.

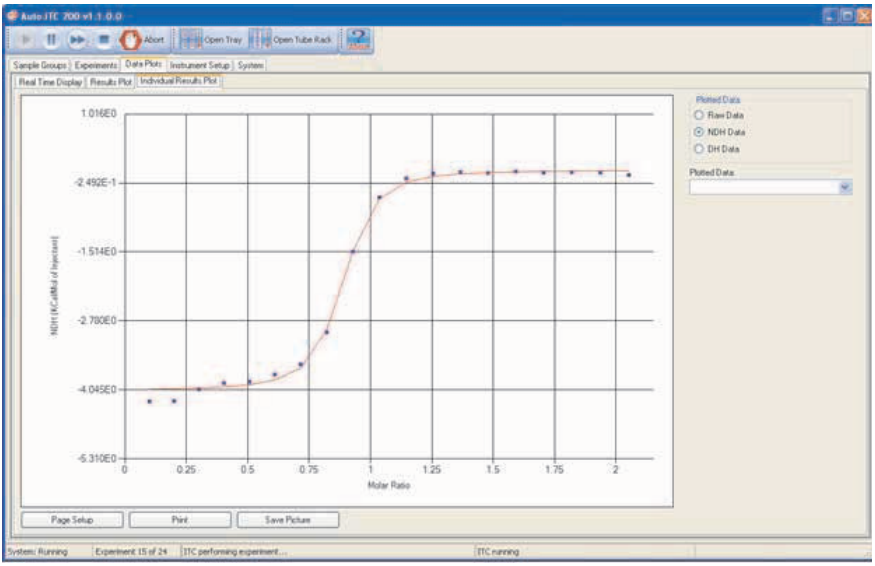


Figure 4-15. Individual results plot tab.

4.7 Automated data analysis

The Excel output spreadsheet contains all the run parameter selections, plus the resulting data, for each run. Different tabs within the Excel sheet contain the runs analyzed by that method; one tab is for control runs, one for one-site model runs, and one for certain setup parameters.

For a titration run where ***Control*** has been selected, the run parameters will be stored in the ***Control*** tab of the Excel spreadsheet. The spreadsheet will contain columns with the mean energy and standard deviation of the peak areas. This provides an analysis of the consistency of the peak sizes, as well as a measure of the blank heat of injection. When the mouse hovers over mean energy column, an embedded graph will pop up, showing the raw data. Moving the mouse slowly from the top of the column down to the bottom will make each graph appear in turn. The standard deviation column contains pop-ups of scatter plots of the area under each peak.

For a titration run where "One-site" model was selected, the run parameters and analyzed data will appear in the ***OneSite*** tab of the Excel spreadsheet. The fitted n, K_D, and ΔH are shown, as well as the calculated Gibbs free energy and entropy. The offset referred to here is the offset used during analysis to adjust the final injections heats to zero. When the mouse hovers over the stoichiometry column, a graph of the raw data will appear. The automated baseline is shown in red. The K_D column contains an embedded graph of the normalized ΔH plot, with the fit curve in red. If a variable is outside the range set in the ***Setup*** tab, the entire spreadsheet line will appear highlighted in red.

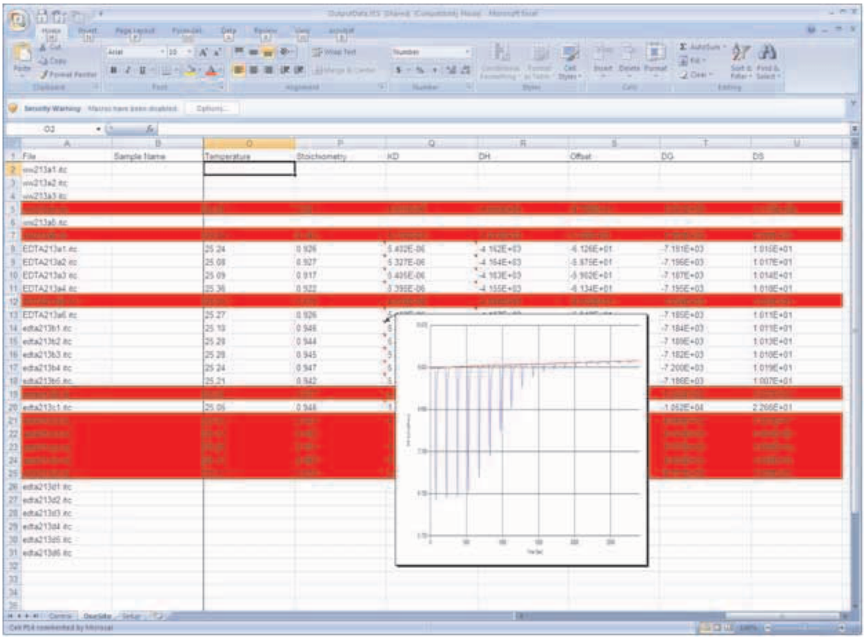


Figure 4-16. Excel output spreadsheet.

4.8 Experimental design

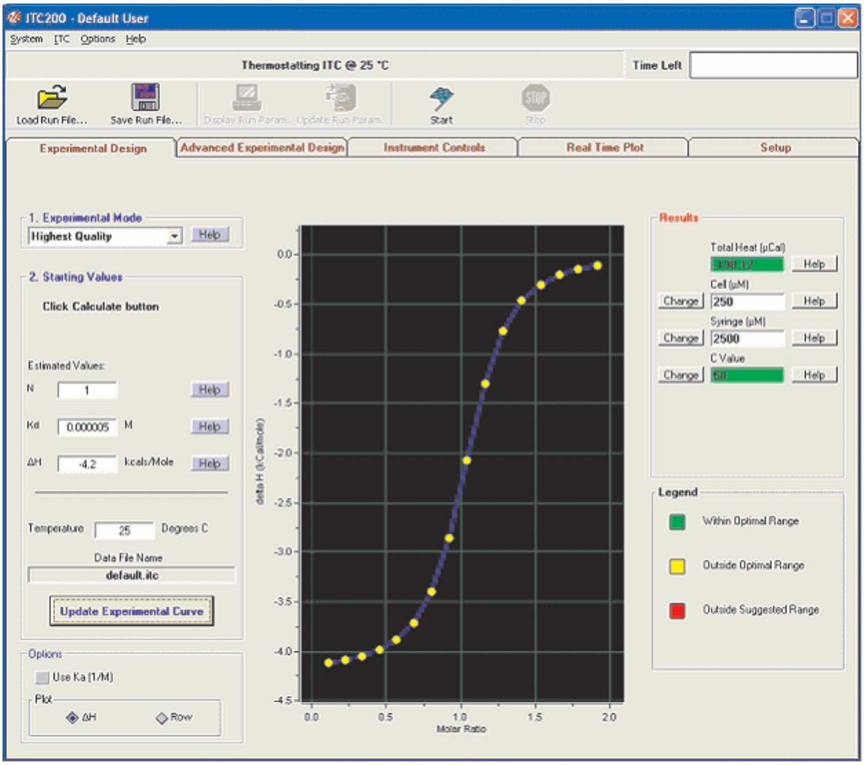


Figure 4-17. Experimental design tab.

When the software is first started, by default, the ***Experimental Design*** tab is selected; this contains the simple run controls. ***Experimental Mode*** can be ***Highest Quality***, ***Minimum Protein***, or ***High Speed***. ***Highest Quality*** uses 20 injections.

These parameters should produce data that is clear and easier to fit. ***Minimum Protein*** uses fewer injections, only 10. The result of these parameters will be the use of the least amount of sample necessary for a successful titration. The ***High Speed mode*** will do one single longer injection (***Single Injection Mode***, SIM).

The expected n, K_D, and ΔH and the desired run temperature will allow the software to calculate the recommended concentrations for the cell and syringe, and set the run parameters based on mode chosen.

If the user is unsure of the K_D for their system, clicking the ***Help*** button causes the software to prompt for the type of compound in cell and syringe. It will then make a guess as to the K_D. The user will still be required to choose values for ΔH and n.

Click the ***Update Experimental Curve*** button to calculate the results. The simulation window will update with a graph, and the ***Results*** column at the right of the screen will have values for the cell and syringe concentrations. The calculated C value is listed below; its background is color-coded.

The C-value predicts the shape or sigmoidicity of the curve. Optimal values for C are between 5 and 500 (green); values between 1 and 5, and 500 and 1000 should work but may not give the best result (yellow). C values less than 1 or greater than 1000 will probably not yield usable data (red).

The user may adjust the two experimental concentrations by using the ***Change*** buttons beside each concentration box.

Any warnings, such as heats too high for the instrument to measure, will appear in the status bar near the top of the screen. It is highly recommended that the users look carefully at the projected curve and make sure that the shape and rough values are reasonable before proceeding.

A pair of options at the bottom of this tab allows users to work in K_D or K_A, ($K_D = 1/K_A$) and to choose whether to view the simulation plot using raw heat per injection (DH) or the heat normalized to the molar ratio (NDH).

4.9 Instrument controls

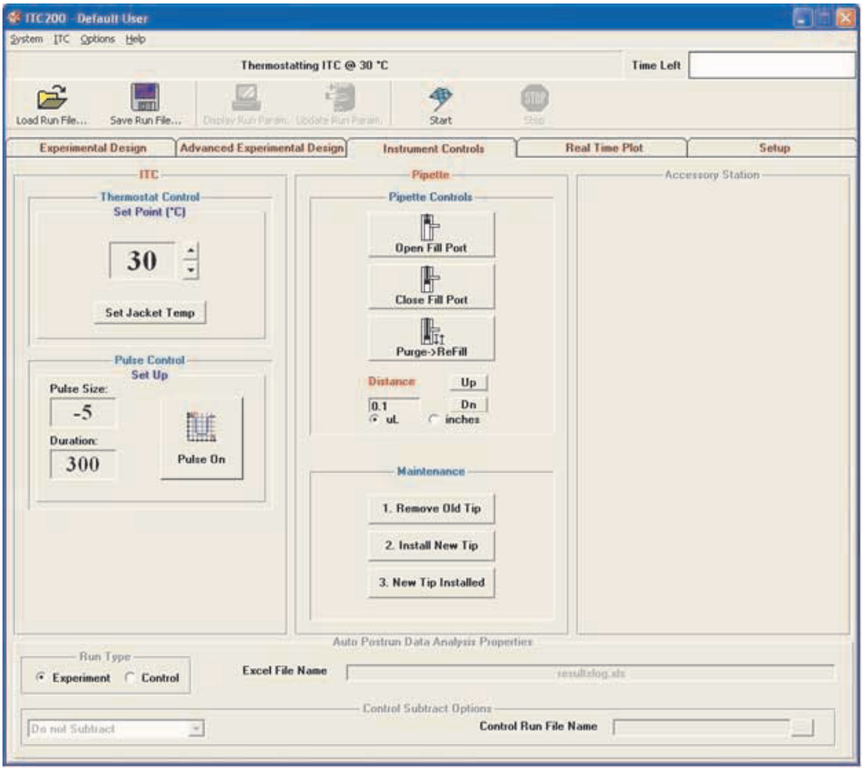


Figure 4-18. Instrument controls tab.

The ***Instrument Controls*** tab contains the controls for direct operation of the instrument. At the top of the window, the user can ***Start*** the run, using whatever parameters are currently present in the ***Experimental Design*** or ***Advanced Experimental Design*** tabs.

Before clicking this button, it is wise to check that all parameters are correct and that a valid, unique data file name has been entered. The software will double-check with the user before allowing any files to be overwritten. The ***Stop*** button, which is available only during a run, will abort the run immediately.

The ***Thermostat Control*** section allows for setting of the thermostat temperature, which will be maintained during the iTC$_{200}$ thermostatic (idle) state. Pre-thermostatting the iTC$_{200}$ and samples at the run temperature will result in shorter equilibration times.

Also, high temperature thermostatting during cell cleaning can improve the effects of the cleaning. Use the arrow buttons to raise or lower the temperature or click in the text box and type in a new number. Click ***Set Jacket Temp*** to set the temperature.

The ***Pulse Control*** section allows for manually administering a DP calibration pulse.

While this is not the most thorough method of checking the *y*-axis calibration, it is the quickest method. Pulses may be applied any time the DP signal is equilibrated and the resulting deflection used as a crude calibration assessment. If Origin for real-time plotting is enabled, Origin will calculate the error. If this is greater than 1%, please see Section *5.2* (Y-axis calibration check) for a more thorough check of the DP calibration.

4.10 Procedures after a run

The MicroCal Auto-iTC$_{200}$ was designed to have its power on for extended periods of time. This will keep the system electronics at the normal operating temperature. It is recommended that the power of the MicroCal Auto-iTC$_{200}$ be turned off during extended periods of down time, such as holidays and vacations. The system will automatically clean itself after each run, so it will be left clean.

NOTICE
For quick start up leave instrument on.

5 Maintenance

This section provides the user with information on the proper maintenance of the instrument to ensure proper function.

The maintenance procedures described here may be performed by the user. In addition, annual preventative maintenance should be performed by a trained GE Healthcare service technician. The technician will inspect the instrument for wear or damage, replace any parts that require it, check the calibrations, and generally ensure that the instrument is working as well as possible.

WARNING!
This instrument uses potentially lethal high voltages that can cause injury and death. There are four areas where extra precautions should be taken when servicing the instrument. These include the power input module, the power supply, the power connector to the calorimeter and the touch screen display.

WARNING!
This instrument is designed with interlocks to prevent operation when the door is open. If these interlocks are disabled and/or any of the cabinet's panels are removed there is a potential for pinch, puncture and electrical injury. Only trained service personnel should service this instrument under these circumstances

CAUTION
Be aware of potential pinch points when the instrument is powered up. The system can move at any time and could potentially cause injury.

CAUTION
The tray drawer and tube drawer both have potential to cause injury by pinching a body part between the face plate and the instrument's frame.

The maintenance methods described below do not require working anywhere near the exposed hazard areas.

5.1 Refilling the reference cell

The iTC$_{200}$ has two cells, the sample cell and the reference cell. The autosampler cleans and refills the sample cell for each run. The reference cell must be refilled manually, approximately once a week. While the system is idling, click the ***Open Door*** button on the touchscreen. The left door will pop open several inches. Swing it further open, until it is out of the way. The iTC$_{200}$ is now accessible through the open door.

To reload the reference cell, gently insert the glass Hamilton syringe into the (right) reference cell until it touches the bottom. Pull up on the plunger until bubbles are being pulled from the cell, and there is no more liquid. Remove and empty the syringe. Clean the syringe if necessary. Pull approximately 300 µL of degassed, distilled water into the syringe, and tap the syringe glass so that all air is at the top volume of the syringe, as bubbles in the cell will cause problems.

After removing the bubbles, insert the syringe into the cell and gently touch the bottom of the cell with the tip of the syringe needle. Raise the needle tip about 1 mm off the bottom of the cell, and hold it there until finished filling. Do not raise the syringe during the filling process. Slowly inject solution into the cell until it spills out the top of the cell stem. Finish the filling with several small abrupt spurts of solution to dislodge any bubbles in the cell. Finally, lift the tip of the syringe to the cell port (just below the visible portion of the cell port) and find the ledge that is formed where the cell stem meets the cell port. Place the syringe on the ledge at the top of the metal cell stem and remove the excess solution. Remove the syringe, and close the left door and make sure it latches.

5.2 Y-axis calibration check

It is recommended that the y-axis calibration be checked every few months to ensure accurate data acquisition. The automatic calibration check routine will send a series of pulses to the cell heaters, dissipating a known power. The offset in the DP as a result of this power is analyzed in comparison to the correct DP offset. It is recommended that Origin for real-time data be enabled for this check.

First use the ***Prep For Baseline*** button in the ***System*** tab to fill the cell and pipette with water and move the pipette into position for a run.

To begin the y-axis calibration check procedure select iTC$_{200}$ software menu ***ITC:Start ITC Calibration Run:Y Axis Check***.

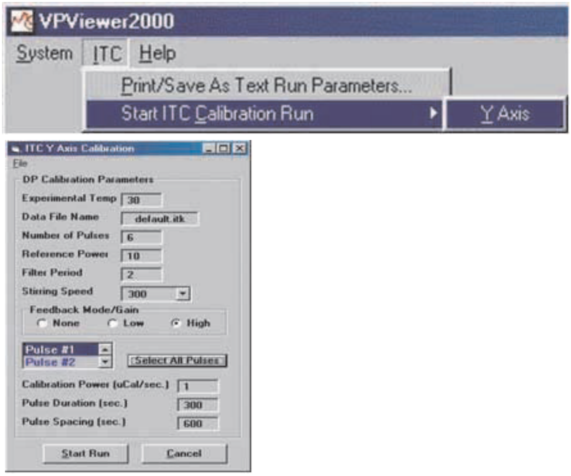


Figure 5-1. Menus

Once the menu has been selected, the Calibration Pulse Setup Window will appear. This window allows the calibration pulses to be modified. Individual pulse parameters are entered by first selecting a pulse or multiple pulses, then entering the desired parameter value into the pulse parameter boxes (Calibration Power, Pulse Duration and Pulse spacing). Users are encouraged to simply use the default y-axis calibration parameters. After the run and pulse parameters are entered, clicking on the ***Start Run*** button will start the run. The ITC will equilibrate in the same manner as it would during a titration experiment.

If creating customized calibration parameters, users must be aware of the DP range limits when setting reference power and pulse sizes. The reference power must be low enough to allow all pulses without hitting saturation, and high enough to allow all pulses without crossing zero. If a pulse size is too small, it can show abnormally high error.

After the final equilibration phase has completed, the initial delay will begin and the pulses will be applied as entered. As each pulse completes, Origin will analyze the pulse region and determine the deflection of the baseline as well as the energy (area) of the pulse. The requested power and energy will also be displayed as will a percent error for both power and energy. The reported error in deflection or energy should be less than 1%. If the error is reported as higher than 1%, please contact GE Healthcare.

For a more rigorous analysis, once the calibration is done and the system is thermostatting again, open the ITC Calibrations project. Click on the ***Y-Axis Calibration (DP, uCal/sec)*** button. Origin will ask for the DP check file. Select the data file just created and click ***Open***. The computer will pause for a few moments. If any of the pulses are out of specifications, a pop-up will inform you. It will ask you to save.

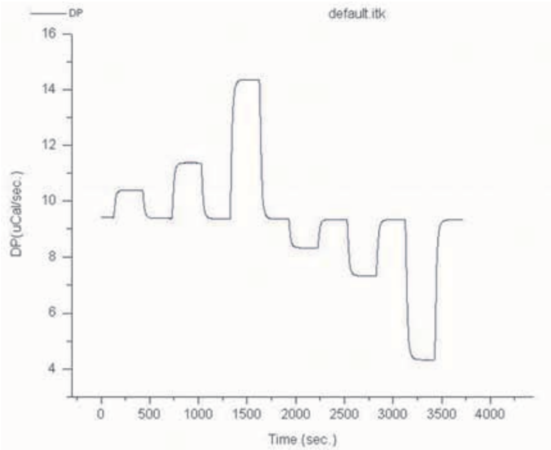


Figure 5-2. Raw data calibration check from y-axis calibration check.

Origin will show four graphs. The upper left graph holds the raw data. The lower two graphs show the energy and power of each pulse. The upper right graph displays the percent error for the energy and power of each pulse. Right-click at the upper right portion of the graph and select ***Go To Window*** from the menu. Check the sizes of the errors. If any of the errors is greater than 1%, please contact GE Healthcare.

5.3 Temperature calibration check

It is recommended that the ITC temperature calibration be checked approximately once every 12 months. The measured temperatures should be within +/- 0.2 degrees of the set temperatures in the iTC$_{200}$ software. This procedure requires a Control Company digital meter and submersible probe or equivalent. The Control Company meter is model #9612; the probe is model #4021. The meter's calibration should be maintained to +/- 0.05 °C.

Make sure the cells have been cleaned, and use the ***Fill Cell*** button in the ***System*** tab of the MicroCal Auto-iTC$_{200}$ control software to fill the sample cell with water. Residue in the cells or empty cells will yield unpredictable results. Open the door, insert the probe into the sample cell and turn the meter on.

1 Use the ***Temperature*** control box under the ***Instrument Controls*** tab in the iTC$_{200}$ software to set the temperature to 30°C.

2 Set the ***Plot Idle Data*** flag so that the current data is displayed.

3 When the instrument is thermostatting at the target temperature, the DP has settled at a negative, stable value, and the DT is 0, use the data reader tool and the

display from the meter to take temperature data points, as close together as possible.

4 Repeat for the second check point at 70°C. If the error for each is not less than 0.2°C, please contact GE Healthcare for assistance.

It is recommended that users keep a log with the date of the calibration check and the software and meter readings for each temperature point.

5.4 Replacing the pipette

To remove the pipette, click the ***Inspect Pipette*** button within the ***System*** tab. The pipette will swing around to the front where it is easily accessible. Open the door, power off the instrument and shut down the software. Unscrew and unplug the pipette connector; there are two #0-80 hex screws holding it on, which require a .050" hex driver. These screws are recessed into the connector (screws shown in *Figure 5-3*). Loosen the thumbscrew on the side of the pipette arm and CAREFULLY slide the pipette up out of the pipette arm. The needle of the syringe is very easy to bend, so be very careful whenever it is not in the instrument.

To reinsert the pipette, or install a new one, carefully slide the pipette into the clamp on the pipette arm and tighten the thumbscrew. The cable should be just forward of the thumbscrew on the arm (see photo below). Plug in the pipette connector and tighten the screws back down. Close the door, reboot the instrument, and restart the software. The system will home again when it starts a run, and the pipette arm will move back into position.

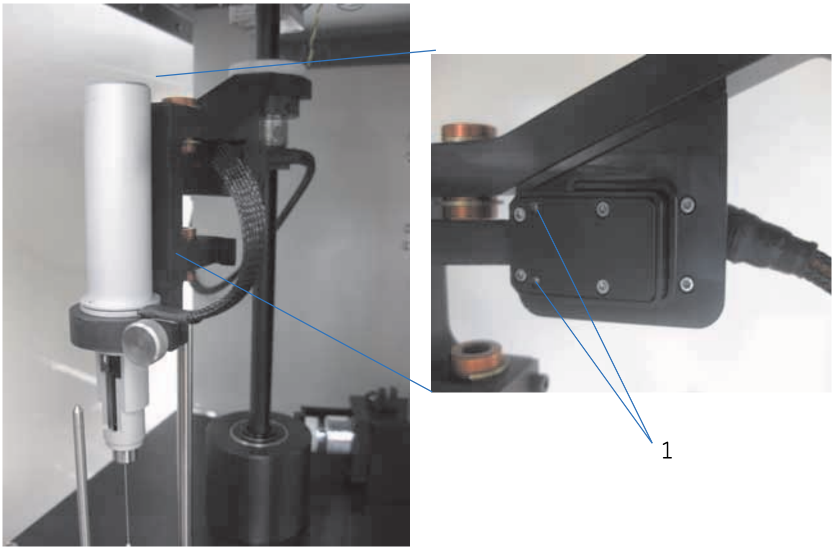


Figure 5-3. Pipette in autosampler; detachment screws (1).

5.5 Replacing the titration syringe

To remove the titration syringe, click the ***Inspect Syringe*** button within the ***System*** tab. The pipette will swing around to the front where it is easily accessible. It will spin for several seconds to make it easy to tell if the needle is bent. If the needle is bent, the end of it will appear to wobble as it spins.

Loosen and then remove the bottom nut on the pipette. The soft-grip tweezers can be used to help grip the syringe without damaging it. Hold the exposed glass of the syringe firmly and pull straight down, pulling the syringe down out of the pipette (See *Figure 5-4*). Carefully put the syringe in a place where it will not roll or be damaged, preferably in the syringe storage case.

To reinsert the titration syringe, or install a new one, the slot at the top of the glass must be lined up with the metal pin in the side of the pipette (See *Figure 5-5*). Gently push the syringe straight up into the pipette. If it is not aligned, the syringe will not go all the way in; take it back out and line it up again. Replace and tighten the bottom nut. The system will home again when it starts a run, and the pipette arm will move back into position, or the user can click the ***Home System*** button in the ***System*** tab.

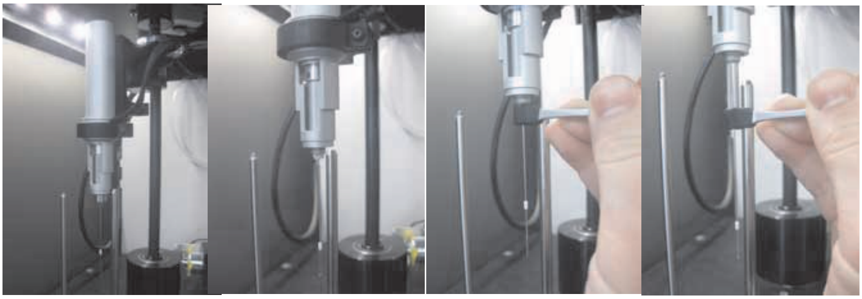


Figure 5-4. Replacing the titration syringe, 1-4.

Figure 5-5. Replacing the titration syringe, 5-7.

5.6 Replacing a cannula

The Titrant Transfer and Cell arms both have metal cannulas that can become clogged, bent, or damaged. The source of the damage should be determined before the cannula is replaced to avoid damaging other cannulas. The advanced user may choose to replace a cannula themselves. Use a cannula provided with the instrument or contact GE Healthcare for a replacement. The cell arm cannula is about 6.5" (17cm) long and the titrant transfer arm cannula is about 5.5" (15cm) long; using the incorrect cannula will cause the system not to work correctly and may cause damage to the instrument.

Figure 5-6. Replacement cannulas.

To replace the cannula, first use the ***Replace Cell Arm Cannula*** button or the ***Replace Transfer Arm Cannula*** button to move the arms to a convenient location for replacing the cannula.

Once the arm is in position, open the door to the MicroCal Auto-iTC$_{200}$, and pull up on the edge of the tubing at the top of the cannula to disconnect it. Loosen the setscrew, using a .050 inch hex driver, in the bottom piece of the holder, below the arm. Slide the cannula up out of the holder and remove it.

To reinsert the cannula, or install a new one, slide it down from the top into the slot in the arm (See *Fig 5-7* below).

For the cell arm, turn the holder so that the set screw is facing you. Slide the bottom piece of the holder up around the cannula, as high as it will go, and tighten the setscrew. On both cannulas, the two set screws should be lined up.

Slide the end of the plastic tubing onto the metal tubing at the top of the cannula. The system will home again when it starts a run, and the arms will move back into position.

It is a good idea to observe the first cleaning and loading cycle to ensure that nothing has been knocked out of alignment.

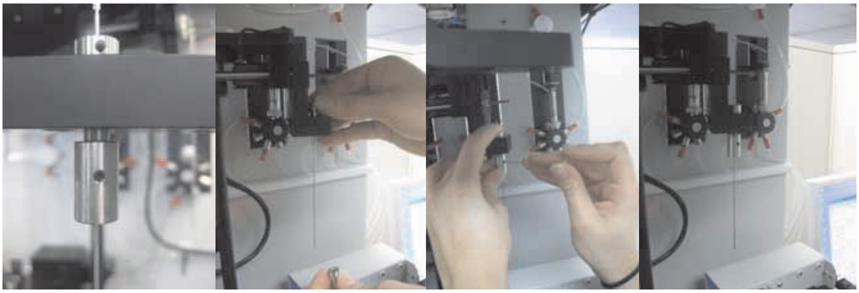


Figure 5-7. Replacing cannulas, 1-4.

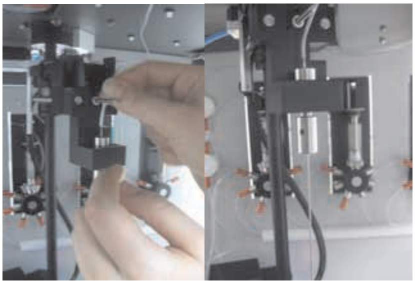


Figure 5-8. Replacing a cannula, 5-6.

5.7 Replacement of fuses

> **WARNING!**
> Always disconnect power from the instrument before replacing fuses.

> **WARNING!**
> Replace fuses ONLY with fuses of same type and rating. Several spare fuses are provided with the original shipment and the power receptacle is labeled with the correct type.

The iTC_{200} and MicroCal Auto-iTC_{200} each contain two fuses, found in the power receptacle at the rear of the instrument, below the power switch and above the plug. If the fuses repeatedly blow, unplug the instrument and contact your local GE Healthcare representative.

6 Troubleshooting

This section contains tips and information for troubleshooting the MicroCal Auto-iTC$_{200}$. Many problems in instrument loading and cleaning show characteristic baseline and titration abnormalities, and knowing which system is affected can greatly speed the resolution of the issue. Some problems can easily be corrected by the average user; some may be corrected by the more advanced user, and some require the expertise of a GE Healthcare service technician. The GE Healthcare service department is happy to provide any advice, parts, or service that may be necessary.

6.1 How to get help

Please contact us for any instrument or data analysis questions or issues you may have. For contact information for your local office, please visit: www.gelifesciences.com/contact, or for MicroCal-specific information, visit: www.gelifesciences.com/microcal.

When e-mailing for technical assistance, if possible, please attach a recent data file(s) (*.itc, raw ITC data file) that demonstrates the problem. Also, please include all details that may be relevant to the problem. For instance, where the problem or question relates to post run data analysis, it is best to attach both the raw data file (*.itc) and the Origin project file (*.opj) and/or Excel (*.xls) spreadsheet generated during data analysis.

There are two general categories of troubleshooting for the MicroCal Auto-iTC$_{200}$ and its operation. The most extreme category is when a system is not working at all. Problems that prevent users from operating the instrument require immediate consultation with a GE Healthcare technician.

Please see the troubleshooting chart. If this does not address the issue, please contact a GE Healthcare service representative.

Customers should not attempt to repair the hardware or software unless instructed to do so by a GE Healthcare service representative.

The second, and less extreme general category of a problem is when a iTC$_{200}$ instrument is functioning, but is not operating within its normal performance specifications. Large baseline drifting, non-repeatable control peaks (water/water) and/or an increase in short term noise level are examples of performance problems. Please see troubleshooting chart in *Table 6-1*.

These problems may be corrected by the operator in most cases. For these types of performance issues it is recommended that customers carry out the following minimum diagnostic steps prior to requesting service:

1 Run a thorough cleaning routine.

2 Load one row in a 96-well plate with 400µL of degassed distilled water.

3 Set up a run of 6 identical titration experiments, each with a minimum of 20, 1µL injections of water into water.

4 Start the run. If possible, observe the cleaning and loading routines.

If, after completion of the steps listed above, the ITC performance is not corrected, please contact the service department for help. The water runs should be provided to the GE Healthcare service technician for evaluation. Following the evaluation, a representative from the service department will contact you with comments and recommendations.

Zip support files

The zip support files utility gives the user a simple way of preparing MicroCal Auto-iTC$_{200}$ files for transfer to GE Healthcare service. These may be helpful to a technician diagnosing a problem remotely. Each button prepares a different type of system file: the ***System Files*** button zips the system settings files; the ***Log Files*** button zips the general system error logs; and the ***Serial Logs Files*** button zips the low-level communication logs. Depending on the type of problem you are experiencing, a service technician may ask for one or more of these. All three buttons create a zip directory with the relevant files, using the filename that is entered in the text box to the right of the button. The zipped directories are found at C:\Support Files, and may be e-mailed or otherwise transferred to the GE Healthcare service technician.

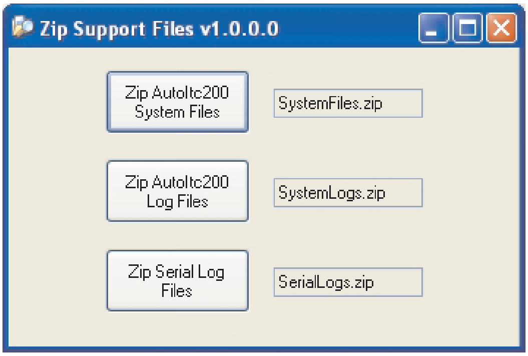


Figure 6-1. Zip support files.

Troubleshooting chart

Table 6-1. Troubleshooting chart.

Problem	Action
Instrument not running; Touchscreen dark	Check that power is plugged in and turned on
Instrument not running; touchscreen shows GE logo	Check that the USB cable is properly connected
	Check that both pieces of control software are running and properly initialized

Problem	Action
Software reports networking errors	Unplug network port from controller, reboot, and reload the control software
Instrument not working properly	Visually check that the cannulas and syringe are straight, nothing is obviously dripping, and sample containers are properly inserted
	Watch a cleaning and loading cycle for any obvious problems
	Check that the fill port adapter that connects to the titration syringe is not damaged; watch it dock during a cleaning and loading cycle
Control software reports initialization errors, communication problems, or hardware errors not covered in this manual	Contact your GE Healthcare service representative
Data shows problems	Check that the nitrogen supply is properly attached; if using a bottle, check that it is not empty
	Check that the reagent bottles are not empty and are properly attached to the correct port
	Refill the reference cell (see *Section 5.1* for instructions)
	If these steps do not resolve the problem, see *Section 6.1* “How to get help for MicroCal instruments”
Cannula is bent	See *Section 5.6* for replacement instructions
Titration syringe is damaged	See *Section 5.5* for replacement instructions

6.2 Too-large peaks

There are several causes of over-large peaks. When running a standard sample, it is clear that all the peaks are larger than they should be, and the final peaks never drop to the normal blank heat of injection level. The baseline is usually within 1 µCal/sec below the reference power, which is its normal level. Running an unknown sample, the large final peaks and unusual binding parameters are the only signs.

This can be caused by a buffer mismatch between the titrant and cell material. A standard water/water or $CaCl_2$-EDTA run should show normal results. If all samples are affected, the most likely cause is that the methanol used for cleaning and drying the titration syringe and cannulas is not being completely removed, which means that not enough nitrogen is being applied. Check that the nitrogen regulator shows at least 20 PSI pressure (i.e., the tank is not empty and the valves are open), and that the pressure line has not been snagged or kinked. It is also possible for there to be a pressure leak within the autosampler cabinet.

In the series of runs below, the nitrogen tank was not turned on for the first run, and the extreme heat from each injection causes the signal to hit negative saturation. The nitrogen was turned on during the cleaning before the second run, which is much better but still shows the extreme heat signature of the residual methanol. The “bounce” after each injection is typical when the signal goes below 0. In the third run, the methanol was properly and completely dried, and the normal baseline noise is visible between the tiny water into water injection peaks.

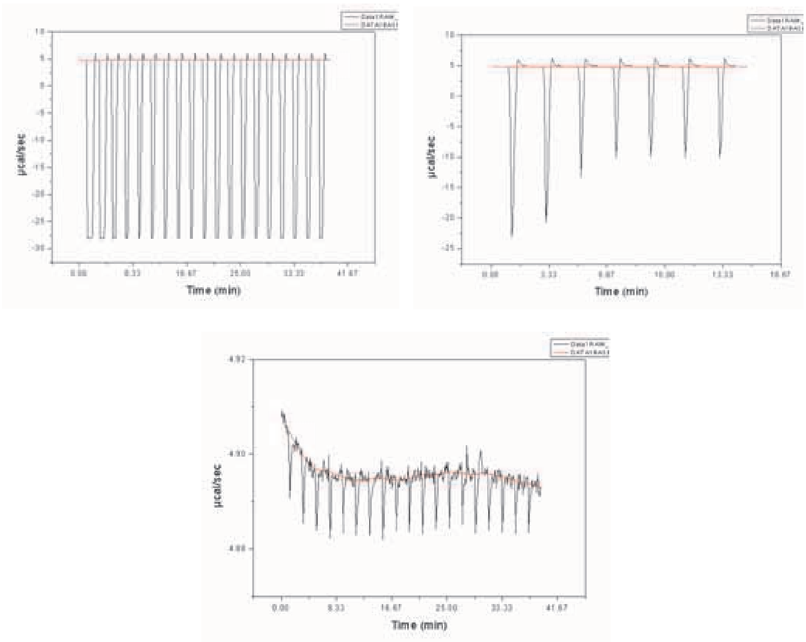


Figure 6-2. Methanol residue data.

6.3 Stepping baseline

This almost always means an empty or underfilled titration syringe. The baseline will start in the normal range, within 1µCal/sec below the reference power, but after each (slightly over-large) injection, the baseline will step down. This is caused by the syringe injecting air into the cell, which creates a disturbance and then shifts the heat capacity of the sample cell, offsetting the baseline.

Check that the correct well in the 96-well plate was filled. A small amount of liquid should be left in the plate after loading, and should still be visible. Check that the lid above the well has been punched; if it is not, there is a problem with the titrant transfer arm. If the pipette has been replaced since the last run, it may not be properly initialized. It is also possible that the fill port dock that connects to the titration syringe is not communicating properly, or that the small fill port adaptor tip that fits into the fill port in the syringe is damaged. With all the arms parked, open the doors and examine the tip. If it is damaged, please contact GE Healthcare. Shut down the MicroCal Auto-iTC$_{200}$ and all software and restart the system; this should reset the communications and take care of any communications problems.

The two titration runs below demonstrate two instances of this problem; the first shows a continuously dropping baseline, while the second is less regular but shows the same characteristic baseline drops.

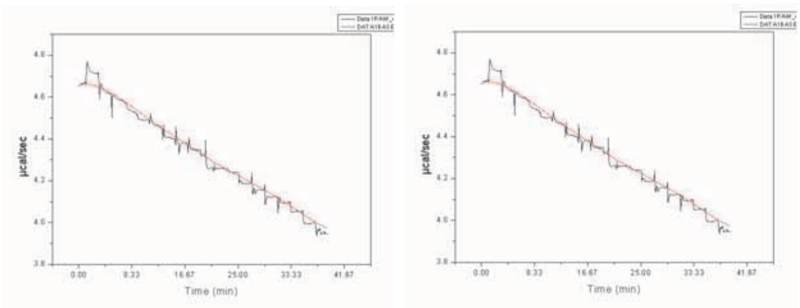


Figure 6-3. Empty syringe data.

6.4 Reversed peaks

This is a rather strange-looking condition in which the baseline starts flat and the peaks initially look normal, but partway through the run, the peaks start to shrink too quickly and then begin going in the opposite direction. The baseline may start low, and it begins to drift slightly as the peaks reverse direction.

This is caused by an underfilled sample cell. Note: it is also possible for an endothermic reaction, with a large buffer mismatch, to have negative initial peaks and positive later peaks. If the sample was taken from the 96-well plate, check that the correct well in the 96-well plate was filled. A small amount of liquid should be left in the plate after loading, and should still be visible. Check that the lid above the well has been punched; if it is not, there is a problem with the cell arm. If the sample was taken from the tube rack, make sure that the proper tube still has sample in it. If the problem persists, carefully watch the cell loading for one autosampler cycle. The sample should not shift in the tubing or drip during loading; if it does, air is leaking into the lines.

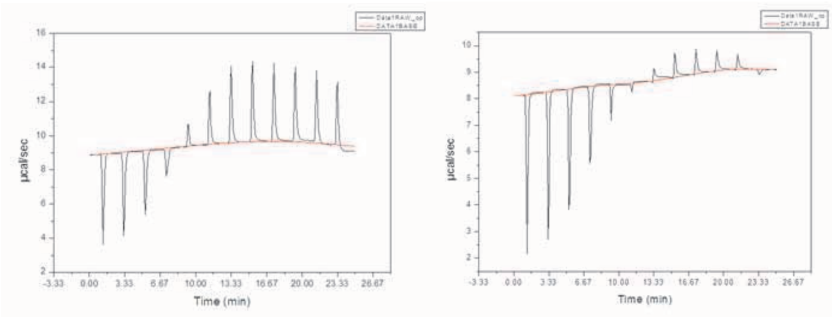


Figure 6-4. Excess heat of injection data.

6.5 Baseline blips

This is a relatively minor situation, where a small, short “blip” occurs in the data. It usually happens at the edge of an injection, and is caused by a bubble in the titration syringe being injected into the cell and rising to the top of the stem. It may cause difficulties with the automated baseline fitting, and may indicate sample degassing occurring in the syringe, a worn-out plunger tip, or a loose fitting allowing bubbles into the tubing. It is common when the samples have not been degassed. By itself, it is not a major concern.

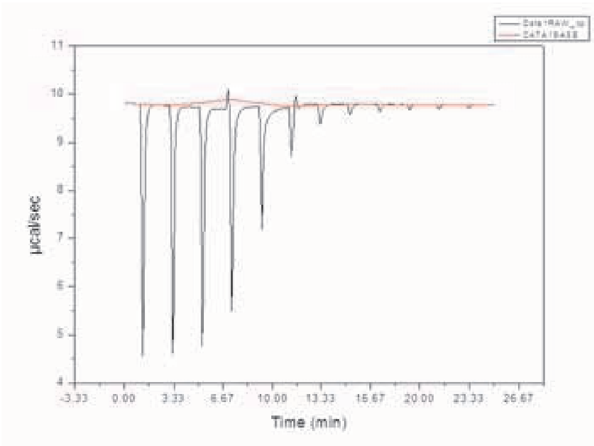


Figure 6-5. “Blip” data.

6.6 Low baseline

This condition appears as a baseline that settles more than 1 µCal/sec below the programmed reference power and frequently indicates an empty or underfilled cell. If the sample was taken from the 96-well plate, check that the correct well in the 96-well plate was filled. A small amount of liquid should be left in the plate after loading, and should still be visible. Check that the lid above the well has been punched; if it is not, there is a problem with the cell arm. If the sample was taken from the tube rack, make sure that the proper tube still has sample in it. Try another titration to see if the problem reappears.

The other common cause of a low baseline is a bent paddle on the titration syringe; the bent paddle causes excessive turbulence, which creates excess heat in the sample cell. The syringe should be checked and replaced, if necessary, (see Section *5.5*) and returned for straightening.

7 Reference information

7.1 Instrument specifications

Performance specifications

Characteristic	Data
Operating Temperature Range	2°C to 80°C
Sample Storage Temperature Range	4°C to 30°C
Response Time	10 seconds
Cell Design:	200 µL, coin-shaped
Titration Syringe:	40 µL
Maximum Usable Volume:	38 µL
Smallest Injection Size:	0.1 µL
Stirring Rate	500 to 1500 RPM

Physical specifications

Description	Data
Cell Material	Hastelloy® Alloy C-276
Dimensions	Calorimeter: 17 × 16 × 36 cm, (6.5 × 6.6 × 14") Controller: 15× 36 × 41 cm, (6 × 14×16") Monitor: 38 × 40 × 20 cm, (15 × 15.5 × 8") Autosampler: 61 × 76× 58 cm, (24× 30 × 23")
Calorimeter weight	6 kg/13 lbs
Controller	9 kg/20 lbs
Monitor	5 kg/10 lbs
Autosampler	90 kg/200 lbs

For operation, the MicroCal Auto-iTC$_{200}$ with a controller requires about 1.2 meters of normal bench space (ca. 70 cm wide).

Electrical specifications for calorimeter and autosampler only

Characteristic	Calorimeter	Autosampler
Voltage	100 to 240 Volts AC	
Frequency	50 / 60 Hz	
Power	70 Watts	300 Watts
Fuses (2)	(2) 4 A, 250 V, Time Delay	(2) 5 A, 250 V, Fast acting
Output	Secondary/Data connection only	
Protective Earth Terminals	Internal/external marking	Internal marking
Mode of Operation	Continuous	
Classification	Class I	

Environmental conditions

Condition	Characteristic	Limits
Operation	Temperature	10°C to 28°C, constant to 2.5°C
	Humidity	≤ 70% RH
Storage (no liquid in cells)	Temperature	-40°C to 70°C
	Humidity	≤ 90% RH

7.2 Ordering information

For ordering information, visit: www.gelifesciences.com/microcal

For local office contact information, visit
www.gelifesciences.com/contact

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