Malvern

LABEL-FREE BINDING ANALYSIS

MICROCALORIMETRY

MICROCAL **PEAQ-ITC** USER MANUAL

MICROCAL PEAQ-ITC
USER MANUAL

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Head office:

Malvern Instruments Ltd.
Enigma Business Park,
Grovewood Road,
Malvern,
Worcestershire WR14 1XZ
United Kingdom.

Tel + [44] (0)1684-892456

Fax + [44] (0)1684-892789

CONTENTS

1 INTRODUCTION

This section contains important user information, description of safety notices, regulatory information and a general description of the intended use of MicroCal PEAQ-ITC, and a list of associated documentation.

The following topics are covered in this section:

About this manual

Purpose of this manual

The *User Manual* provides you with the instructions needed to operate and maintain the product. This is a complement to *MicroCal PEAQ-ITC Operating Instructions*.

Scope of this document

This manual covers MicroCal PEAQ-ITC including the MicroCal PEAQ-ITC Control Software.

Typographical conventions

Software items are identified in the text by bold italic text. A colon separates menu levels, thus File:Open refers to the Open command in the File menu.

Hardware items are identified in the text by **bold** text (e.g., Power switch).

Text entries that MicroCal PEAQ-ITC Control Software generates or that the user must type are represented by a monospaced typeface (e.g., C:\MicrocalITC).

Important user information

Note: All users must read the entire *Operating Instructions* before installing, operating or maintaining the product.

Always keep the *Operating Instructions* at hand when operating the product.

Do not operate the product 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 of the product

MicroCal PEAQ-ITC is an Isothermal Titration Calorimeter system designed for bio-molecular interaction studies in research applications.

MicroCal PEAQ-ITC is intended for research use only and shall not be used in any clinical procedures or for diagnostic purposes.

MicroCal PEAQ-ITC is not suitable for operation in a potentially explosive atmosphere or for handling flammable liquids.

WARNING! Do not operate the product in any other way than described in the *MicroCal PEAQ-ITC user documentation*.

Prerequisites

In order to operate MicroCal PEAQ-ITC safely and according to the intended use, 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 chemical and biological materials.
- You should understand the concepts of isothermal titration calorimetry.
- You must read and understand the Safety instructions of these *Operating Instructions*.
- The system must be installed according to the instructions in Installation.

Safety notices

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.

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 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 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.

Regulatory information

Introduction

This section lists the directives and standards that the MicroCal PEAQ-ITC system fulfills.

Manufacturing information

The table below summarizes the required manufacturing information. For further information, see the EC Declaration of Conformity (EC DoC) document.

Name and address of manufacturer	Malvern instruments Grovewood road Malvern Worcestershire WR14 1XZ, United Kingdom

Conformity with EU Directives

This product complies with the European directives listed in the table, by fulfilling the corresponding harmonized standards.

A copy of the EC Declaration of Conformity is available on request.

Directive	Title
2004/108/EC	Electromagnetic Compatibility (EMC) Directive
2006/95/EC	Low Voltage Directive (LVD)

CE marking

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

- used as a stand-alone unit, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from Malvern Instruments, except for alterations described in the user documentation.

International standards

The standard requirements fulfilled by this product are summarized in the table below.

Standard	Description	Notes
EN 61010-1, IEC 61010-1, UL 61010-1, CAN/CSA C22.2 No. 61010-1	Safety requirements for electrical equipment for measurement, control, and laboratory use.	EN standard is harmonized with EU directive 2006/95/EC.
EN 61326-1, IEC 61326-1 (Emission according to CISPR 11, Group 1, class A)	Electrical equipment for measurement, control and laboratory use - EMC requirements.	EN standard is harmonized with EU directive 2004/108/EC.

Instrument safety compliance specifications

MicroCal PEAQ-ITC calorimeters carry the CUE (Canada, USA, Europe) mark:

The CUE mark (authorized by TÜV America, a division of TÜV Süddeutschland) signifies that:

- The instrument has been tested by an accredited certification body and meets applicable Canadian electrical safety standards/requirements (CSA/SCC).
- 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).

Regulatory compliance of connected equipment

Any equipment connected to MicroCal PEAQ-ITC should meet the safety requirements of EN 61010-1/IEC 61010-1, or relevant harmonized standards. Within EU, connected equipment must be CE marked.

Associated documentation

Introduction

This section lists the user documentation that is delivered with MicroCal PEAQ-ITC and related literature that can be downloaded or ordered from Malvern Instruments.

User documentation

The user documentation for MicroCal PEAQ-ITC consists of:

- *MicroCal PEAQ-ITC Operating Instructions*
- *MicroCal PEAQ-ITC User Manual*
- *MicroCal PEAQ-ITC Analysis Software User Manual*

Related literature

Additional downloadable material can be found at: www.malvern.com

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SYSTEM DESCRIPTION

This section provides a description of MicroCal PEAQ-ITC and an overview of its components.

The following topics are covered:

General introduction

MicroCal PEAQ-ITC provides detailed insight into binding energetics.

The system has a sample cell and provides direct measurement of the heat absorbed or evolved as a result of mixing precise amounts of reactants. The sample and reference cells are made from Hastelloy™, a highly inert material.

Data analysis is performed using MicroCal PEAQ-ITC Analysis Software, wherein the user obtains the stoichiometry (n), dissociation constant (K_D), and enthalpy (ΔH) of the interaction. The MicroCal PEAQ-ITC Analysis Software can also be used to fit more complicated models.

Isothermal titration calorimetry

Introduction

Isothermal Titration Calorimeters (ITC) measure the heat change that occurs when two substances interact. Heat is liberated or absorbed as a result of the redistribution of non-covalent bonds, for example, when the interacting molecules go from the free to the bound state.

An ITC mixes the binding partners and monitors the heat changes by measuring the power required to maintain zero temperature difference between the reference and sample cells.

The reference cell usually contains water, which has the same heat capacity as most of the sample buffers. The sample cell contains:

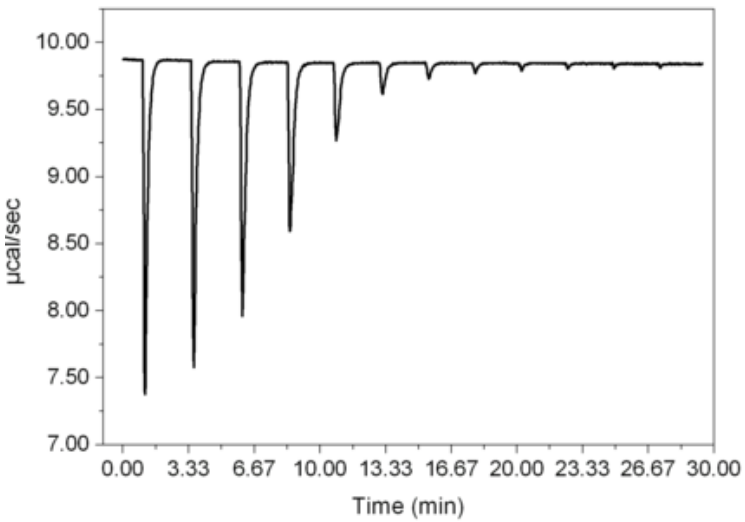
- one of the binding partners (often, but not necessarily a macromolecule), and
- a stirring syringe, which holds the other binding partner (often, but not necessarily a ligand).

Experimental procedure

Typically, the ligand is injected into the sample cell, in 2 to 3 µl aliquots, until its concentration is two- to three-fold greater than that of the sample cell material. Each injection of the ligand results in a heat signature that is first integrated with respect to time

and then normalized for concentration. This titration curve is fitted to a binding model to extract the affinity (K_D), stoichiometry (n) and the enthalpy of interaction (ΔH) (also called heat of reaction).

The following illustration shows an example experimental curve.



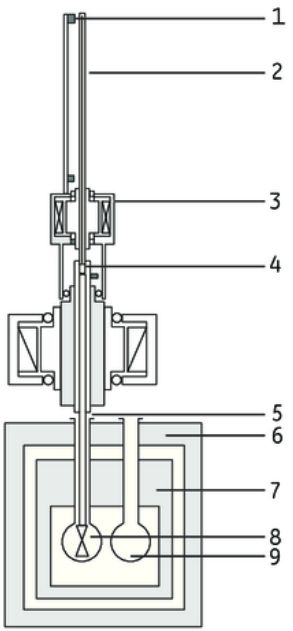
Notice that the first injection results in a larger deflection from the baseline, denoting a larger heat and nearly 100% binding. At the conclusion of the experiment, very little of the injected substance binds, resulting in little or no deflection (heat change) from the baseline.

Also, notice that the value on the y-axis decreases upon binding. This is the power needed to keep the sample cell at the same temperature as the reference cell.

Heat is given off during the reaction, therefore less power is required to compensate the temperature differences. This is characteristic of an exothermic reaction. In contrast, an endothermic reaction results in spikes rising from the baseline and hence, more power is required to compensate the temperature differences.

Main components of an ITC

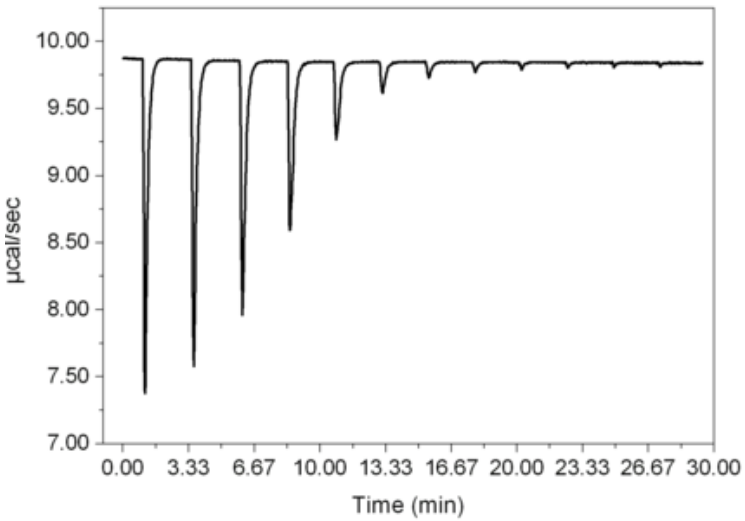
The following illustration shows the main components of an ITC instrument.



Part	Description
1	Sensor
2	Lead screw
3	Injector
4	Plunger
5	Syringe
6	Outer shield
7	Inner shield
8	Sample cell
9	Reference cell

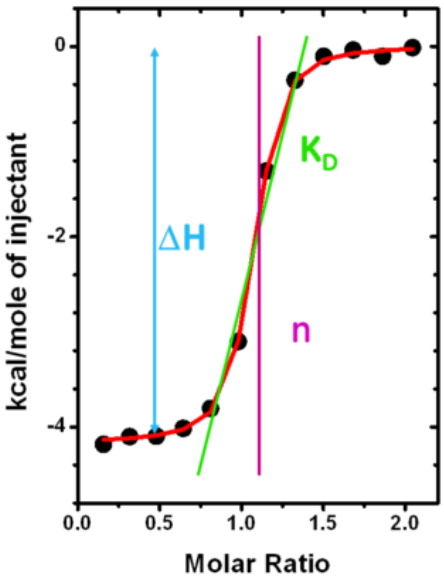
Raw data

The temperature difference between the sample cell and the reference cell is converted to power and directly read out as raw data. An example of this is shown in the following illustration. Each spike, followed by a return to the baseline, is an injection.



Injection heat

The individual injection heat is calculated by integrating the raw data (power) from each injection over time. The following figure shows each individual injection heat, normalized by the amount of titrant injected, as a function of the molar ratio of titrant/cell material in the sample cell. The fitted curve of a 1:1 binding model is overlaid in red. A general illustration of how the thermodynamic parameters n, K_D, and ΔH are related to the titration curve is also overlaid.



In the case of this simple 1:1 binding experiment, the enthalpy is directly measured/fitted as the heat of 100% binding. The stoichiometry is intuitively denoted by the midpoint of the titration, between 100% binding and 0% binding. The steepness of the rise to saturation is related to binding affinity. For any given system, the steepness of this region is also directly related to the sample concentration.

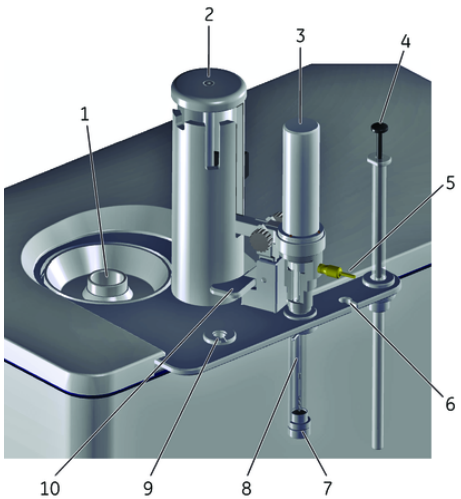
System overview

The following illustration shows the MicroCal PEAQ-ITC instrument with the washing module and the Controller PC.

Part	Description
1	MicroCal PEAQ-ITC washing module
2	MicroCal PEAQ-ITC cell unit
3	Controller PC (not shown)

Instrument cell unit

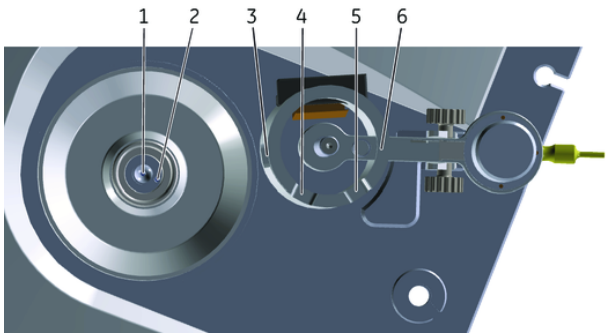
The following illustration shows the MicroCal PEAQ-ITC cell unit.



Part	Description
1	Sample cell
2	Injector tower
3	Pipette
4	Loading syringe
5	Fill Port Adaptor (FPA)
6	FPA Storage Location
7	Wash/load station
8	Titration syringe
9	Titration loading station
10	Clamp

Injector tower (top view)

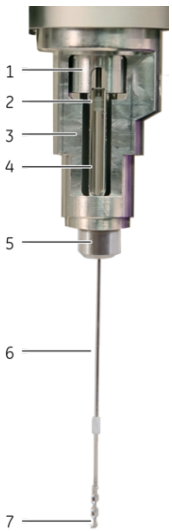
The following illustration shows a top view of the injection tower of the MicroCal PEAQ-ITC cell unit.



Part	Description
1	Sample cell
2	Reference cell
3	Cell Location
4	Rest Location
5	Load Location
6	Clean Location

Pipette

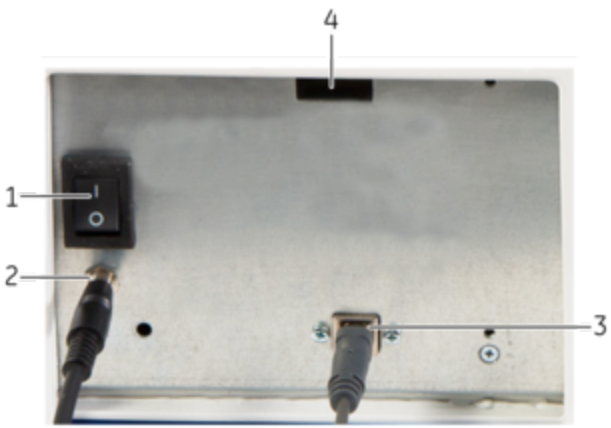
The following illustration shows the MicroCal PEAQ-ITC pipette unit.



Part	Function
1	Rotating assembly
2	Plunger tip
3	Pipette housing
4	Syringe glass
5	Retaining nut
6	Syringe paddle stem
7	Syringe tip

Connections at the rear of the cell unit

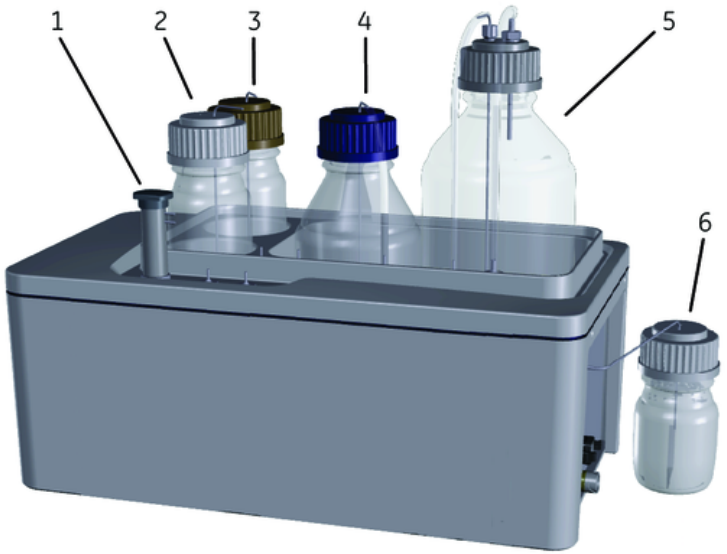
The following illustration shows the connections at the rear of the MicroCal PEAQ-ITC cell unit.



Part	Function
1	Power switch
2	24 VDC input from power supply (AC plug)
3	USB connection to Controller PC
4	Fan

Washing module

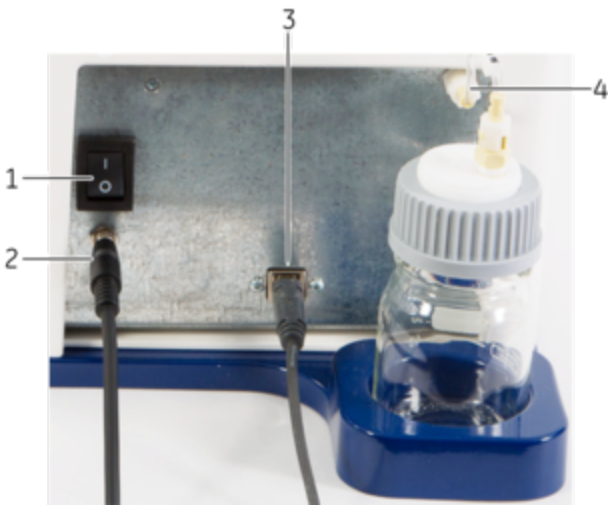
The following illustration shows the MicroCal PEAQ-ITC washing module.



Part	Function
1	Cell Cleaning Tool
2	Detergent bottle
3	Methanol bottle
4	Water bottle
5	Waste bottle
6	Overflow bottle

Connections at the rear of the washing module

The following illustration shows the connections at the rear of the MicroCal PEAQ-ITC washing module.



Part	Function
1	Power switch
2	24 VDC input from power supply (AC plug)
3	USB connection to Controller PC
4	Waste/overflow bottle connection

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INSTALLATION

This section provides brief information about the installation of MicroCal PEAQ-ITC.

The following topics are covered in this section:

Note: Any equipment connected to the MicroCal PEAQ-ITC must fulfill applicable standards and local regulations.

Software installation

Introduction

The instrument is delivered with preinstalled MicroCal PEAQ-ITC Control Software. Contact your Malvern Instruments service representative if the control or operation software reports initialization errors, communication problems, or hardware errors not covered in this manual.

Controller PC

For proper function of the Controller PC, the following conditions must be ensured:

- Each component of the software installation must be performed with full *administrative* privileges.
- If users without local administrative privileges will be operating the system, the administrator must modify their access rights in order to assure proper operating environment for them.
- **User Account Control (UAC) Settings** must be set to **Never Notify**.
- `Port 8090` must be opened for TCP traffic on localhost.
- **Windows Update** must not be set to automatically install updates.
- **System Restore** must be disabled.
- The `C:\MicrocalITC` directory's **Security** must be set to **Full control** for the user's group.
- The `MicrocalITC.exe` must be run as administrator.

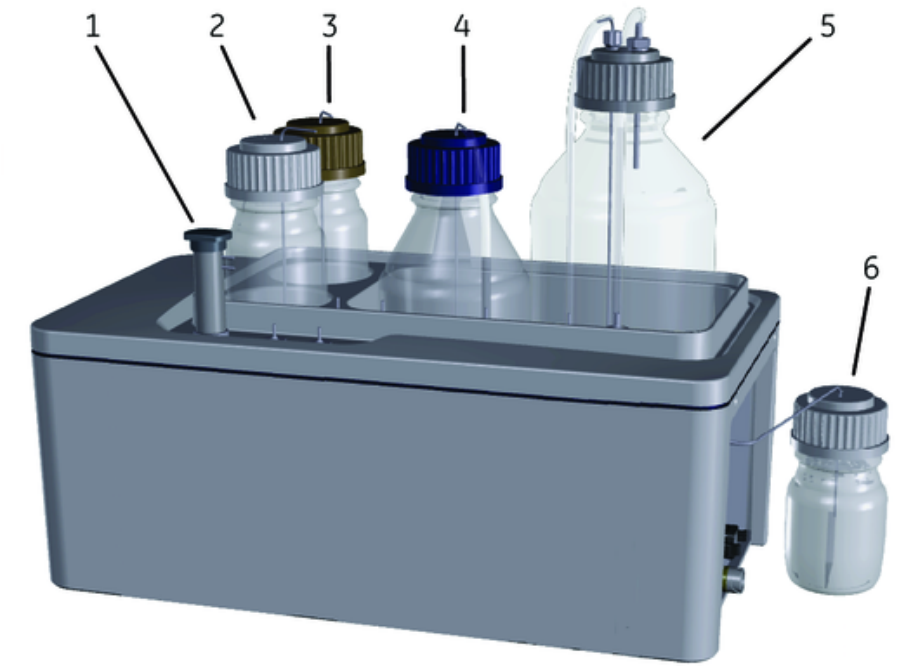
Bottle preparations

Introduction

Before running an experiment for the first time, you may need to perform one or all of the following tasks:

- Fill bottles
- Empty the overflow bottle
- Prime tubing

Washing module bottles



Part	Function
1	Cell Cleaning Tool
2	Detergent bottle Recommended detergents: 20% Contrad™ 70 or 14% Decon™ 90.
3	Methanol bottle ≥ 99% pure methanol ("HPLC Grade" is recommended)
4	Water bottle
5	Waste bottle
6	Overflow bottle

Fill the bottles

Although the bottles do not have to be full before you begin a procedure, you should make sure that there is in enough cleaning fluid each bottle to perform the required procedure.

To fill the bottles, follow the steps described in the following table:

Step	Action
1	Verify that the system is in an idle state.
2	Unscrew the plastic cap of the bottle by turning it counter-clockwise. 
3	Fill bottle using standard lab procedures.
4	Tighten the cap by turning clockwise until snug.

Empty the waste bottle

Follow this procedure to empty the waste bottle.

Step	Action
1	Verify that the system is in an idle state.
2	Unscrew the grey cap of the waste bottle by turning the lid counter-clockwise.
3	Empty the bottle according to your laboratory waste handling procedures.
4	Reattach the cap by turning it clockwise until it is snug.

Note: If the waste bottle is full, the excess will fill the overflow bottle and shut down the vacuum pump.

Prime the tubing

Prime the tubing from the bottles to the washing module to make sure that the full volume of cleaning fluid is delivered. This procedure is required only if the tubing from the bottles to the washing module have been drained of fluid and contain air.

Step	Action
1	Make sure all bottles contain enough cleaning fluid and all fluid lines are connected.
2	Start an instrument cleaning cycle using the rinse cell and rinse syringe cleaning methods. See Clean the instrument.

When the tubing has been primed (visibly clear of air bubbles), the system uses the majority of the remaining procedure time to dry the syringe. This occurs with approximately eight minutes remaining in the procedure. You can let the procedure finish or click **Cancel** at this time.

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CONTROL SOFTWARE

This section describes the MicroCal PEAQ-ITC Control Software that is delivered with the MicroCal PEAQ-ITC. The user interfaces are also described in detail. MicroCal PEAQ-ITC Control Software controls the calorimeter. See Operation for instructions on how to operate the MicroCal PEAQ-ITC.

The following topics are covered:

Overview

Software description

The MicroCal PEAQ-ITC is delivered with two software components as outlined in the following table.

Software component	Description
MicroCal PEAQ-ITC Control Software	This software is used to control MicroCal PEAQ-ITC and is preinstalled on the Controller PC.
MicroCal PEAQ-ITC Analysis Software	This software is supplied for data analysis. Accessed via the MicroCal PEAQ-ITC Control Software. For more information see *MicroCal PEAQ-ITC Analysis Software User Manual*.

MicroCal PEAQ-ITC Control Software

Part	Area	Displays ...
1	Header	• Notification icon • **HELP** icon • **ABOUT** icon

Part	Area	Displays ...
2	Primary navigation bar	• **Run Experiment** command button • **Maintenance** command button • Design Experiment command button • **Connection** status • Settings icon • Font size slider
3	Secondary navigation bar	... varying choices, dependent on which command button is active in the primary navigation bar (e.g. **Browse** for method or experiment file, set experiment parameters, workflow status, etc.)
4	Workspace	... single or multiple views with intuitive options to run and design experiments and also maintain the instrument.
5	Instrument control bar	... instrument control center for direct manipulation of temperature, syringe, cell. etc.

Start the MicroCal PEAQ-ITC Control Software

The MicroCal PEAQ-ITC Control Software is used to control the MicroCal PEAQ-ITC instrument. The software and hardware need to be started in sequence for correct initialization.

To start the MicroCal PEAQ-ITC Control Software, follow the steps described below:

Step	Action
1	Start the computer and log in to Windows.
2	Turn on the MicroCal PEAQ-ITC instrument using the Power switch at the rear of the unit.
3	Start the MicroCal PEAQ-ITC Control Software. *Result*: The MicroCal PEAQ-ITC Control Software is launched.

Common controls

Introduction

This section describes buttons and labels available in all workspaces.

Notification icon

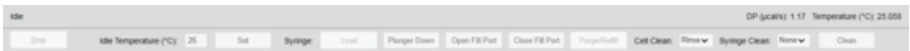
Notifications are displayed in the top of the MicroCal PEAQ-ITC Control Software window. Notifications are generated by the software when there is important information you need to attend to.

Click the Notification icon to display a list of critical warnings (red), cautions (orange), notices (green) and information (blue) that require your attention.

Alert	Description
	**Critical**. Shown for example when: • cautions regarding instrument maintenance has been ignored • the instrument or part of the instrument is offline • connection to the operating software is not established The number in the icon indicates the number of alerts. Thus, this example indicates six critical alerts.
	**Caution**. Shown for example when: • information regarding instrument maintenance has been ignored • Windows System Restore is enabled or Windows update is set to Scheduled Installation
	**Notice**. Shown when there is nothing to inform about.
	**Information**. Shown for example when it is time to perform instrument maintenance.

Instrument control bar

The experienced user can control the instrument from the instrument control bar located at the bottom of the MicroCal PEAQ-ITC Control Software window.



The instrument control bar can be used to execute all loading and cleaning tasks performed by the instrument. This allows the user to perform loading and cleaning tasks without referring to the software guided workflows.

Controls on the instrument control bar

The labels and buttons of the instrument control bar is explained in the following tables.

Label	Displays
Injection	Current injection number.
Remaining Time	Remaining time of the ongoing experiment run.
DP	Current differential power.
Temperature	Current sample cell temperature (°C). Set temperature within parenthesis.

The commands available are as follows:

Command	Description
Stop	Click to stop the current instrument activity.
Temperature:	Enter a temperature (°C) in the Temperature: box. Click Set to change the sample cell's target temperature.
Load	Click to load the titration syringe with titrant material (only enabled if the plunger is in the down position). **Note**: The pipette must be in Load Location with the Fill Port Adapter attached.
Plunger Down	Click to move the plunger down.
Open Fill Port	Click to move the plunger tip to a position above the fill port. (Only available if the Show Manual Syringe Operations box is selected in the Settings workspace, see Settings)
Close Fill Port	Click to move the plunger tip down so that it blocks the fill port. (Only available if the Show Manual Syringe Operations box is selected

Command	Description
	in the Settings workspace, see >Settings)
Purge/Refill	Click to push the plunger tip all the way down and back up again, to dislodge bubbles, if any, on the inside of the syringe. (Only available if the Show Manual Syringe Operations box is selected in the Settings workspace, see Settings)
Cell Clean:	Click the down arrow to select a cell cleaning method. See Choose cleaning method. **Note**: The Cell Cleaning Tool must be installed in the sample cell.
Syringe Clean:	Click the down arrow to select a syringe cleaning method. See Choose cleaning method. **Note**: The pipette must be in Clean Location with the Fill Port Adapter attached.
Clean	Click to start the selected cleaning method(s).

Help and About

Click the **HELP** button to open the software documentation.

Click the **ABOUT** button to display information on software and license agreement.

Settings

Click the settings button to enter the **Settings** workspace.

Setting	Description
Unit	MicroCal PEAQ-ITC will display measured and calculated energy as Joule or Calories. • To select Joule or Calories, click the down arrow next to **Unit** and then click **Joule** or **Calories**.
Show Manual Syringe Operations	Select the **Show Manual Syringe Operations** box to make the **Open Fill Port**, **Close Fill Port** and **Purge/Refill** buttons available on the instrument control bar.
Experiments	Set what folder to initially browse in the **Experiments** tab of the **Start Exper-**

Setting	Description
	iment workspace.
Folder	• Type the path to the folder in the **Experiments Folder** box or • click the browse button and then navigate to the folder in the **Browse For Folder** dialog.
	Set what folder to initially browse in the **Methods Folder** tab of the **Start Experiment** workspace.
Methods Folder	• Type the path to the folder in the **Methods Folder** box or • click the browse button and then navigate to the folder in the **Browse For Folder** dialog.

When a setting has been changed, an asterisk * will appear next to the name of the setting (e.g. **Display Unit***).

- Click **Apply** to save all changes.
- Click a workspace name to leave the **Settings** workspace.

Font scale slider

Use the slider to change the size of text and icons in the workspace. Move the slider left to decrease the size of text and icons, move the slider right to increase the size of text and icons. Graphs in the workspace will automatically change size to fill available space. That is, decreasing the font and icon size will make graphs larger, increasing the font and icon size will make graphs smaller.

Run Experiment view

Introduction

From the **Run Experiment** view you can open a method, load the instrument, start an experiment and clean the instrument.

- Click **Run Experiment** to enter the **Run Experiment** view.

Load workspace

Click **Load** to enter the **Load** workspace.

The **Load** workspace contains a software guided workflow on how to load the instrument sample cell and titration syringe. The load instrument workflow is described in Load the instrument.

Note: Experienced users can perform loading tasks from the instrument control bar described in Instrument control bar.

Clean workspace

Click **Clean** to enter the **Clean** workspace.

The **Clean** workspace contains a software guided workflow on how to clean the instrument sample cell and titration syringe. The clean instrument workflow is described in Clean the instrument.

Note: Experienced users can perform cleaning tasks from the instrument control bar described in Instrument control bar.

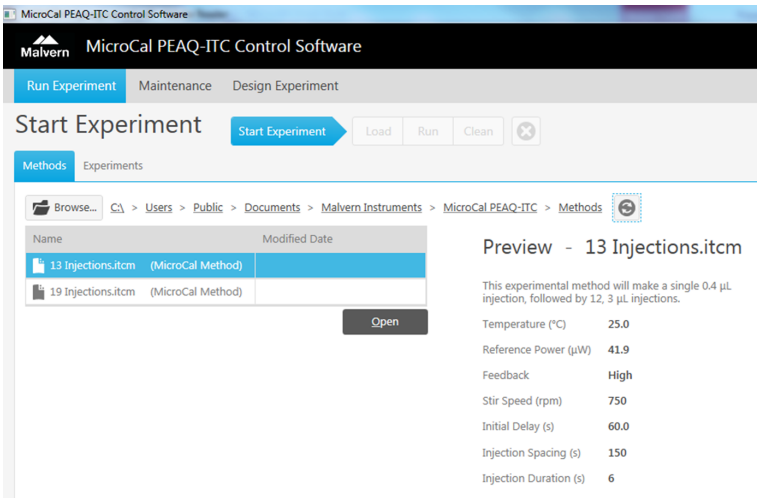
Start Experiment workspace

Introduction

The **Start Experiment** workspace is the first view of the MicroCal PEAQ-ITC Control Software.

The **Start Experiment** workspace contains two panes.

- The left, file list pane, where you select methods and experiments.
- The right, **Preview** pane, where a preview of the selected methods or experiments are displayed.



Open a method or an experiment

- Click **Methods**, browse to a new folder or select a file in the list and then click **Open**

  or

- Click **Experiments**, browse to a new folder or select a file in the list and then click **Open** .

The path to the current folder is visible on top of the file list. Click folder names in the path or in the file list to navigate. Alternatively, click the browse button to open the Browse For Folder window.

Note: The Browse For Folder windows will browse for folders only and not for files.

To refresh the file list, click the **Refresh** button .

Note: Only experiment files are visible when the **Experiments** tab is chosen, and only method files are visible when the Methods tab is chosen.

Note: In most cases the MicroCal standard method can be used.

Preview pane

If you have selected a method, the **Preview** pane will show the experiment parameters associated with the method. If you have selected an experiment, the **Preview** pane will show a list of experiment parameters and a preview of the differential power as a function of time.

Experiment and method files

MicroCal PEAQ-ITC Control Software can open calorimetry data files (experiment files) or method files. Method files only contain instrument settings. Experiment files also contain calorimetric data from an experiment run. Parameters from both file types can be used to create new experiment runs.

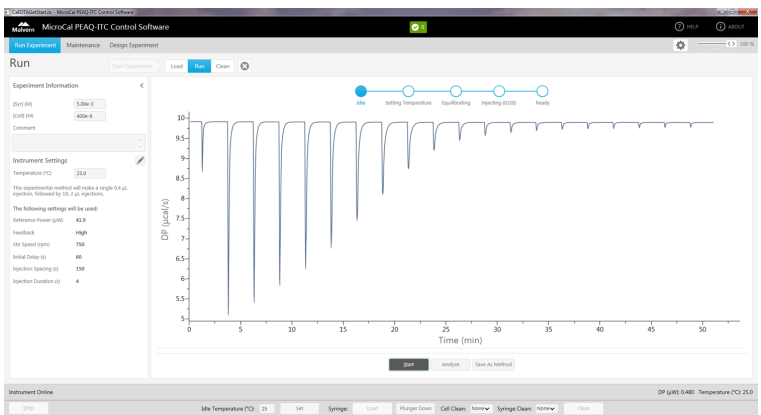
File type	File extension	Description
Experiment file	itc	Calorimetric data from MicroCal PEAQ-ITC.
Method file	itcm	Method file created with MicroCal PEAQ-ITC Control Software or MicroCal PEAQ-ITC Analysis Software. Contains instrument settings only.

Run workspace

Introduction

The **Run** workspace contains two panes.

- The left **Experiment Information** pane where the experiment parameters for the open method or experiment can be edited.
- The right live pane where the running experiment can be monitored in real time.



Experiment Information pane

- To hide the **Experiment Information** pane, click the left arrow ‹.
- To show the **Experiment Information** pane, click the right arrow ›.

The upper part of the **Experiment Information** pane displays sample cell and titration syringe concentrations. The concentrations are used by the analysis software to fit binding models. Instrument settings can be edited under the heading **Instrument settings**.

Experiment information

- Enter titrant concentration in the **[Syr] (M)** box.
- Enter sample cell solution in the **[Cell] (M)** box.
- Enter any comments in the **Comment** box.
- To edit the instrument settings, click the **Edit settings** button

Result: More instrument settings and injection settings become available.

Instrument settings

Instrument setting	Description
Temp (°C)	Enter the desired run temperature for the experiment. The instrument's operating range is 2°C to 80°C. Most runs are performed at 25°C (room temperature).
Reference Power	Enter a value for reference power. The differential power (DP) baseline will equilibrate near this value. The reference power is a small constant amount of power supplied to the offset heater of the reference cell. This causes the DP feedback system to supply compensating power to the sample cell to equilibrate the temperatures. The best choice for the reference power setting can be determined by the anticipated size and sign of the titration heats. The following table gives some guidelines.
FeedBack	The feedback mode affects both response time and sensitivity. High gain provides the fastest response time. No gain (passive mode, None) provides the highest sensitivity. Most ITC reactions require using the High setting.

Expected reaction type	Suggested reference power
Large exothermic	Large value (approx. 10 µcal/s using high feedback)
Large endothermic	Small value (approx. 0.5 µcal/s)
Unknown	Intermediate value (5 µcal/s using high feedback)

Instrument setting	Description
	Monitoring long, slow thermal processes (for example, kinetics, metabolic rates) might benefit from using the None (passive) or Low settings.
Stir speed (rpm)	Enter the sample cell stirring speed in revolutions per minute. Faster stirring may be necessary if the sample cell contains suspended particles, for example agarose beads.
Initial Delay (s)	Enter the time (s) between the start of the run and the first injection (standard value 60 s). This is necessary to establish a baseline before the first injection.

Injection settings

Injection setting	Description
# of Injections	Enter the number of injections for the titration (ITC) experiment. The multiple injection method requires a minimum of 10 to 15 injections. The single injection method uses one injection.
Volume(µL)	Enter the volume (µl) of titrant to be injected from the pipette into the sample cell for the injection(s) selected in the injection list.
Duration(s)	Enter how long (seconds) each injection of titrant into the sample cell for will be.
Spacing(s)	Enter the time (seconds) between the beginning of the injection (s) selected in the injection list and the beginning of the next injection (or end of run). The injection spacing must allow enough time between injections to allow the DP signal to return to the baseline after an injection peak deflection. Typical values for this parameter range from 90 to 180 seconds, depending on the feedback mode, temperature and reaction kinetics. Note: For the single injection method, the spacing should be at least 90 seconds longer than the duration of the injection.

Edit injections

- Double-click a box in the injection list to edit an injection.
- Click **Apply to all** to copy the selected injection's settings to all other injections in the list.
- Click **Apply to Rest** to copy the selected injection's settings to all following injections in the list.

Live pane

When an experiment is running the experiment results are displayed in a graph of differential power as a function of time. Instrument status is displayed in the flow chart above the graph.

Status	Description
Idle	The instrument is waiting for user input.
Setting temperature	The instrument is changing sample cell temperature.
Equilibrating	The instrument is waiting for the DP signal to reach the baseline.
Injecting	The instrument is injecting titrant into the sample cell. Injection number and total number of injections is shown within parenthesis.
Ready	The experiment is ready. The instrument is waiting for user input.

Buttons in the live pane

- Click **Start** to start the experiment.
  Result: The instrument starts running the defined experiment.
- To save experiment information as a method to be used later, click **Save As Method**.
  Result: The **Save As** dialog box opens.
- To open the experiment result in MicroCal PEAQ-ITC Analysis Software, click Analyze.

Result: MicroCal PEAQ-ITC Analysis Software starts.

- To close the the method file and return to the Start Experiment workspace, click the **Close** button located above the flow chart.

Maintenance view

Introduction

From the Maintenance view you can view the software guided workflows for changing the plunger tip, replacing the syringe and cleaning the syringe.

- Click **Maintenance** to enter the Maintenance view.

Note: Experienced users can perform maintenance tasks from the instrument control bar described in Instrument control bar.

Change Plunger Tip workspace

The **Change Plunger Tip** workspace contains a software guided workflow on how to change the plunger tip. The change plunger tip workflow is described in Replace the syringe plunger tip on page 70.

Click **Change Plunger Tip** to enter the Change Plunger Tip workspace.

Replace Syringe workspace

The **Replace Syringe** workspace contains a software guided workflow on how to replace the titration syringe. The replace titration syringe workflow is described in Replace the titration syringe on page 77.

Click **Replace Syringe** to enter the Replace Syringe workspace.

Clean Syringe workspace

The **Clean Syringe** workspace contains a software guided workflow on how to change the plunger tip. The change plunger tip workflow is described inClean the titration syringe on page 81.

Click **Clean Syringe** to enter the Clean Syringe workspace.

Design Experiment view

Introduction

From the **Design Experiment** view you can create method files.

- Click **Design Experiment** to enter the Design Experiment view.

Guided mode

The **Guided** mode of the **Experimental design** view helps you design one-to-one binding experiments and simulate basic runs.

- Click **Guided** to enter Guided mode.

For more information see Guided experimental design.

Advanced mode

The **Advanced** mode of the **Experimental design** view lets you edit more experimental parameters, but you will not receive recommendations or comments as help text.

- Click **Advanced** to enter Advanced mode.

For more information see Advanced experimental design.

5 OPERATION

This section provides the information required to safely operate the MicroCal PEAQ-ITC.

The following topics are covered in this section:

Preparing the samples

Proper sample preparation is critical for successful ITC experiments. General guidelines for sample preparation will be discussed here. These guidelines use the terminology of binding experiments using biological samples, but may be readily used for other types of samples.

In this section:

- The importance of sample preparation
- Preparing small molecule solutions
- Preparing macromolecule solutions
- Calculating cell concentrations
- Additional notes

The importance of sample preparation

Introduction

Isothermal Titration Calorimetry (ITC) is designed to measure the heat of binding when the titrant, also referred to as the ligand, is injected into the sample cell containing the macromolecule sample material. A single ITC experiment and subsequent analysis can simultaneously determine all binding parameters (n, K, ΔH, ΔS).

Minimizing control heat

When the titrant is injected into the cell material and mixed, some additional heat effects other than the binding heat are detectable. The key for successful ITC experiments is to minimize the control heat, thereby allowing the binding heat to be measured more accurately. This control heat will include both the heat of mixing and the heat of dilution. Two primary sources of large control heats are buffer mismatches between the titrant and the macromolecule sample in the sample cell, and a highly concentrated titrant.

Buffer mismatch

The most common mismatch occurs due to pH differences between the titrant and the macromolecule solution, but mismatch could also be a result of salt concentration, or additives such as dioxane, DMSO, glycerol, etc. and the heat of dilution when high concentration of ligand solution from the syringe is injected into the macromolecule solu-

tion. The heat of dilution will also be small, but may become large for ligands that form aggregates at higher concentration in the syringe. The most important step in preparing an assay is buffer exchange, which can be achieved by dialysis or by gel filtration.

Concentration determination

Accurate concentration determination is very important when running a calorimetric experiment. Errors will have direct impact on the thermodynamic results. Errors in cell concentration directly affect the stoichiometry, have little effect on enthalpy, and mildly affect affinity. Errors in titrant concentration, on the other hand, directly affect both the stoichiometry and enthalpy, and mildly affect affinity.

Preparing small molecule solutions

Introduction

Most small molecule ligands (such as drugs and inhibitors) are supplied in solid form. Solutions can be prepared by dissolving the compound in buffer solution or using organic solvents if the compound has low solubility in buffer solution.

Preparing samples using buffer solution

To prepare samples in buffer solution, follow the steps in the following table:

Step	Action
1	Prepare the buffer solution using distilled water.
2	Dissolve a known amount of the compound in the buffer solution.
3	Check the pH. If the pH of the solution is found to differ from the pH of the buffer solution by more than 0.05 units, the pH should be adjusted with a small amount of HCl or NaOH.

Preparing samples using organic solvents

To prepare samples using organic solvents, follow the steps in the following table:

Step	Action
1	Dissolve the compound in DMSO or some other organic solvent (100 mM or higher).
2	Dilute 50 to 100 fold with buffer. **Note**: Care should be taken to keep the ligand from precipitating when diluted. The concentration of organic additives, such as DMSO, in the final ligand solution should be

Step	Action
	kept as low as possible (to 1% to 2%, if possible; but no more than 5%) since the macromolecule solution requires addition of the same additive at the same concentration in order to minimize the mismatch heats.

Preparing macromolecule solutions

Introduction

Macromolecule solutions should normally be dialyzed against the buffer solution using a dialysis membrane having the proper molecular weight cut off (MWCO). However, a lyophilized macromolecule sample devoid of salts or additives may be dissolved directly into the buffer, and used without dialysis. The pH of the solution should be checked and adjusted, if necessary. Solid macromolecule samples containing salts and additives, should be dialyzed against the experimental buffer.

Preparing macromolecule solution by dialysis

The following steps describe how to prepare a macromolecule solution by dialysis.

Step	Action
1	Dialyze the sample at 4°C against a relatively large volume of buffer solution and at least two changes of buffer. The duration of dialysis depends on the sample and buffer, as well as the membrane used. For example, if glycerol at 10% is added to aqueous buffer solution and a 6000 to 8000 MWCO membrane used, it requires at least one overnight dialysis for glycerol to reach concentration equilibrium in the macromolecule solution.
2	Determine the concentration of the macromolecule after dialysis, and remove particles in the solution by filtration or centrifugation. **Note**: Accurate values for binding parameters depend on precise concentration measurements of ligand and macromolecule in the final solutions.

Alternatively, buffer exchange can also be performed using gel filtration. For more information, contact Malvern Instruments.

Preparing macromolecule solution with an additive

If one of the solutions (e.g., ligand solution) contains an additive such as DMSO, then the same additive at an identical concentration should be added to the other solution (e.g., protein solution) to minimize the heat of mixing. As indicated earlier, the stability of the

macromolecule in the presence of the additive should be determined before proceeding. The pH of all final solutions should be checked after additives are added, and matched within 0.05 pH units.

Calculating cell concentrations

c-value

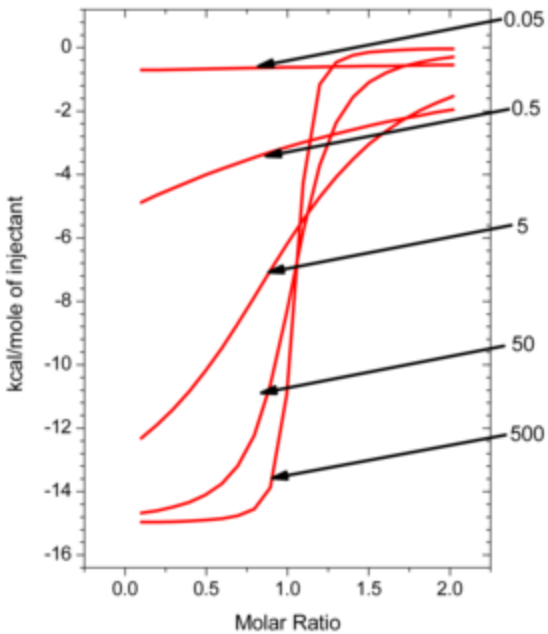
ITC is designed to detect the heat that is absorbed (endothermic) or liberated (exothermic) when two solutions containing the binding partners are mixed. The appropriate concentration of the sample material in the sample cell, usually a macromolecule, will depend on the binding affinity, number of binding sites, and heat of binding, ΔH. The following equation (Wiseman T. *et al.*, Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Anal. Biochem.***179**, 131-137 (1989)) is used when designing ITC experiments to determine the appropriate sample concentration or c value.

$$c = n \cdot M_{tot} \cdot K_A = \frac{n \cdot M_{tot}}{K_D}$$

Parameter	Description
c	sample concentration/affinity-related parameter, should lie between 1 and 1000 (preferably 10 to 500 when solubility, availability of material or the sensitivity of the instrument is not limiting)
n	binding stoichiometry (the number of ligand binding sites on the sample molecule)
M_{tot}	molar concentration of sample molecule in the cell
K_A	association equilibrium constant
K_D	dissociation equilibrium constant

Sample concentration limitations

The following figure depicts simulated curves of the same macromolecular system run at different c-values.



There may be practical limitations that affect the choice of sample concentration:

Experiments including...	should be studied at...
high affinity interactions (low K_D)	low concentrations. (The minimum concentration that will typically cause a confidently measurable heat change for a 1:1 interaction is about 10 µM.)
low affinity interactions (high K_D)	high concentrations. (The concentration that can be used may be limited by availability or solubility of the sample molecule.)

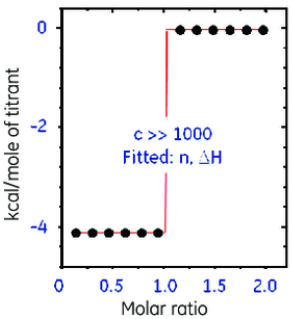
Note: Techniques such as competition experiments or working at high ligand concentration in the case of weak binding can help alleviate these limitations.

Affinity determination at different c-values

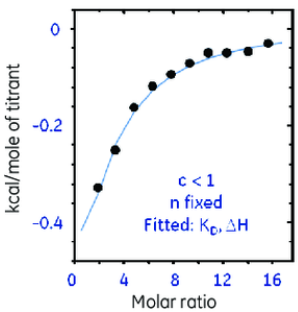
The affinity is poorly determined at high c-values.

At low c-values, one may assume (and not vary) a stoichiometry in the fitting model and inject enough titrant to attain a high molar ratio in order to extract both an affinity and a binding enthalpy.

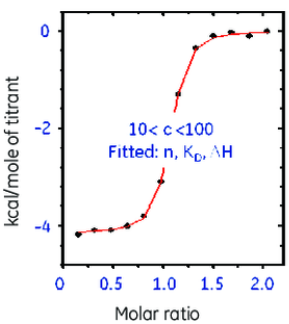
At high c-values



At low c-values



At medium c-values



Additional notes

Calculating syringe concentrations

For a 1:1 binding reaction, the molar concentration of ligand in the injection syringe is typically 10 to 20 times higher than the molar concentration of sample molecule in the cell. This will make sure that the cell material will become saturated or close to saturation by the end of the titration experiment.

Note: Errors in titrant concentration strongly affect the stoichiometry and enthalpy calculations, and mildly affect affinity calculations.

Injection number and duration

The specifications for the MicroCal standard method are presented in the following table.

Parameter	Value
Number of injections	12
Injection volume	3 µl
Initial injection volume	0.4 µl (to minimize the impact of equilibration artifacts sometimes seen with the first injection) **Note**: The data point from this initial injection is discarded before data analysis.
Pipette volume	About 38 µl of ligand solution (sufficient for one typical experiment)

Experimental temperature

It is most convenient to perform ITC experiments at 25°C to 30°C (i.e., slightly above room temperature) unless other factors dictate differently. Since the cells are passively cooled by heat exchange with the jacket, experiments at low temperature require a longer time for temperature to reach equilibrium before injections can begin.

At high temperatures (above 50°C), the baseline becomes noisier, which has an effect on the quality of data. Other factors that influence the choice of the experimental temperature are the binding affinity and the stability and/or solubility of the ligand or sample molecule. Some solutes, particularly proteins, are not stable above room temperature for long periods of time, and in such cases it may be desirable to work at lower temperatures.

To determine the change in heat capacity, ΔC_P, associated with binding, experiments must be performed over a range of temperatures (e.g., 10°C to 40°C) to obtain the temperature dependence of the heat of binding.

Control heat determination

As discussed earlier, a control experiment is required to determine the heat associated with the dilution of the ligand when it is injected from the syringe into the buffer. This experiment will also include contributions from the injection process itself and any other operational artifacts, which can collectively be thought of as the "instrument blank". If heat effects for the control run are small and constant, the average heat of injection can be subtracted from the results of the sample run before curve fitting to obtain binding parameters.

However, large heat effects for the control and heat effects that change as the titration proceeds may indicate mismatch between ligand and sample buffer (see The importance of sample preparation). Buffer matching should then be checked before proceeding with the experiment. If trends in the control results cannot be eliminated by careful buffer matching, they may result from ligand aggregation or self-association in the syringe. More complex evaluation algorithms should be considered in such cases.

Reducing agent

It has been found that the presence of DTT (1,4-dithiothreitol) in solution will often cause a drastic shift in ITC baseline as the experiment progresses. If the presence of a reducing agent is required for protein stability, then β-mercaptoethanol (less than 5 mM) or TCEP (Tris[2-carboxyethylphosphine] hydrochloride; less than 2 mM) should be used rather than DTT.

Reverse titration

Most titrations are carried out with the macromolecule solution in the cell and the ligand solution in the syringe. If both binding partners are macromolecules (or both are small molecules) normally the component with multiple binding sites is placed in the cell.

However, there are instances where it might be advantageous or even necessary to switch the location of the two components and carry out the reverse titration. Examples of such instances are:

- If the component, which normally goes in the syringe has low solubility, it may be easier to use that solution in the cell, where its concentration does not need to be nearly as high.

- If the macromolecule becomes unstable over time in the sample cell, either due to continuous stirring or a high experimental temperature, it may be more stable if placed in the syringe. The solution in the syringe is not stirred or thermostated at experimental temperature until shortly before it is injected into the cell.

Prepare the instrument

Introduction

This section describes the procedures needed to prepare the MicroCal PEAQ-ITC for a run.

Turn on the MicroCal PEAQ-ITC instrument

Once the MicroCal PEAQ-ITC instrument has been connected to the Controller PC, it is ready to use. At the rear of the instrument unit is a power on/off switch that must be in the **on (I)** position.

Software description

The MicroCal PEAQ-ITC is delivered with two software components as outlined in the following table.

Software component	Description
MicroCal PEAQ-ITC Control Software	This software is used to control MicroCal PEAQ-ITC and is preinstalled on the Controller PC.
MicroCal PEAQ-ITC Analysis Software	This software is supplied for data analysis. Accessed via theMicroCal PEAQ-ITC Control Software. For more information see *MicroCal PEAQ-ITC Analysis Software User Manual*.

Start the control software

TheMicroCal PEAQ-ITC Control Software is used to control the MicroCal PEAQ-ITC instrument directly. The software and hardware need to be started in sequence for correct initialization.

To start the MicroCal PEAQ-ITC Control Software, follow the steps described in the following table.

Step	Action
1	Start the computer and log in to Windows.
2	Turn on theMicroCal PEAQ-ITC instrument using the Power switch at the rear of the unit.
3	Double-click theMicroCal PEAQ-ITC Control Software icon.  *Result*: TheMicroCal PEAQ-ITC Software is started.

Leave the power on

During frequent operations, the power may be left on as long as the user interface program, MicroCal PEAQ-ITC Control Software, is running. The software automatically makes sure that the system does not incur any damage and keeps the MicroCal PEAQ-ITC sample cell ready.

Periods of inactivity

When the system will not be used for extended periods of time it is recommended to:

- close the MicroCal PEAQ-ITC application
- switch off the power
- keep both the sample and reference cells filled with deionized water

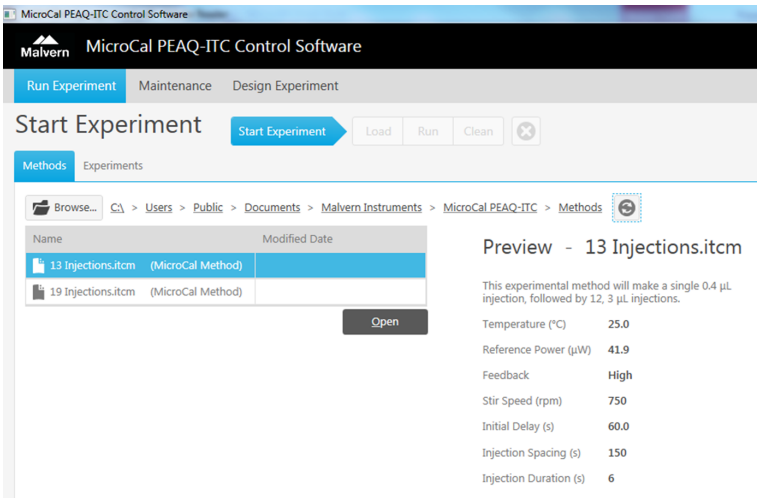
Open a method or an experiment

Introduction

This section describes how to open a predefined method or experiment in the MicroCal PEAQ-ITC Control Software.

Open a method or experiment

In the Start Experiment workspace, open a method file or an experiment file.

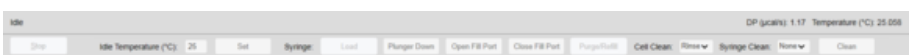


- Click **Methods**, browse to a new folder or select a file in the list and then click Open or
- Click **Experiments**, browse to a new folder or select a file in the list and then click Open.
  Result: The method opens in the Run workspace.

Note: In most cases the MicroCal standard method can be used.

Instrument control bar

The experienced user can control the instrument from the instrument control bar located at the bottom of the MicroCal PEAQ-ITC Control Software window.



The instrument control bar can be used to execute all loading and cleaning tasks performed by the instrument. This allows the user to perform loading and cleaning tasks without referring to the software guided workflows.

For more information, see *MicroCal PEAQ-ITC User Manual*.

Experiment and method files

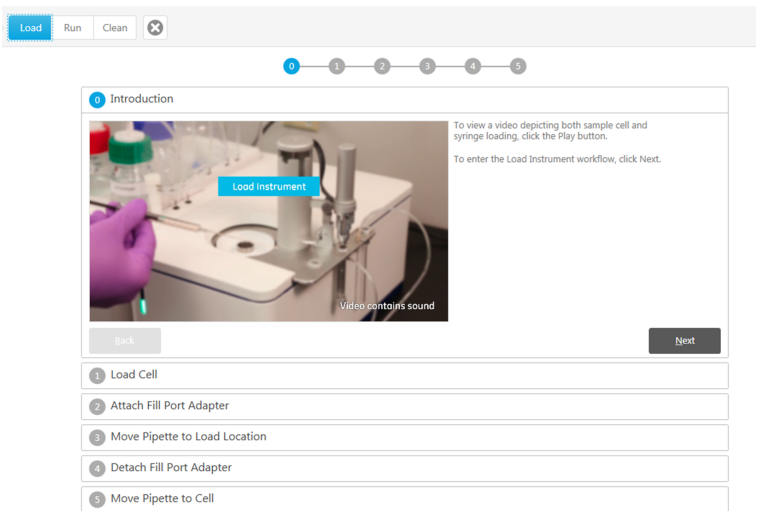
MicroCal PEAQ-ITC Control Software can open calorimetry data files (experiment files) or method files. Method files only contain instrument settings. Experiment files also contain calorimetric data from an experiment run. Parameters from both file types can be used to create new experiment runs.

File type	File extension	Description
Experiment file	itc	Calorimetric data from MicroCal PEAQ-ITC.
Method file	itcm	Method file created with MicroCal PEAQ-ITC Control Software or MicroCal PEAQ-ITC Analysis Software. Contains instrument settings only.

Load the instrument

Load workspace

This workspace is found in the Run Experiment view. Follow the guided workflow that steps the user through loading both the sample cell and the titration syringe. Additionally, the user has the option of watching videos detailing the procedure.

Step	Action
1	Click **Load** *Result:* The Load workspace opens. 
2	To view a instruction video showing both sample cell and syringe loading, click the **Play** button in the instruction video window.
3	To start the Load Instrument workflow click **Next**.

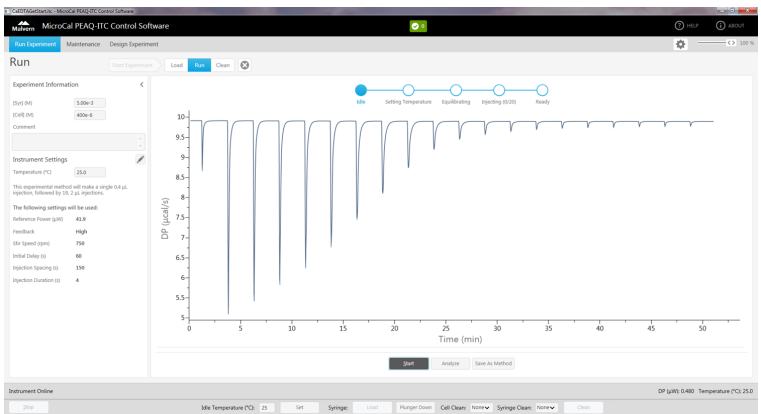
Note: Software-instrument connection is required to load the instrument.

Start the experiment

Introduction

This section describes how to start an experiment. Experiments are started from the Run workspace.

- Click **Run** to enter the Run workspace.



Experiment parameters are displayed in the left Experiment Information pane. Real-time experiment status is displayed in the right pane.

Experiment information

- Enter titrant concentration in the [Syr] (M) box.
- Enter sample cell solution in the [Cell] (M) box.
- Enter any comments in the Comment box.
- To edit the instrument settings, click the Edit settings button

  Result: More instrument settings and injection settings become available.

For more information, see *MicroCal PEAQ-ITC User Manual*.

Run experiment

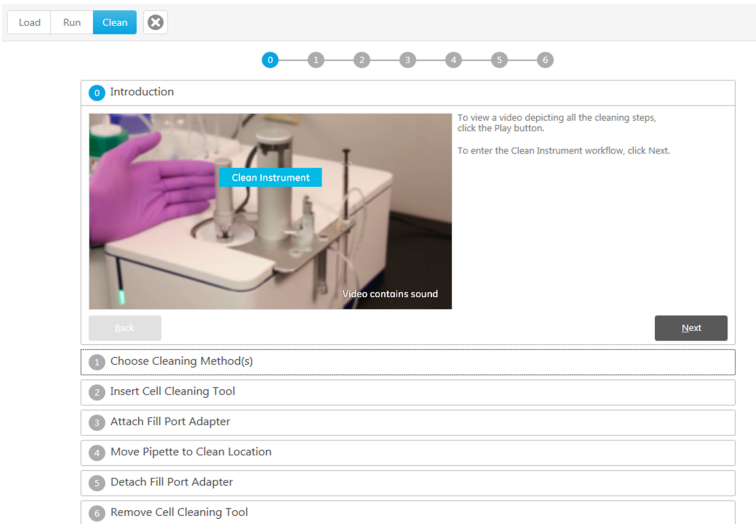
- Click **Start** in the right live pane to start the experiment. The user will be prompted to enter a filename and save location for the .itc file.

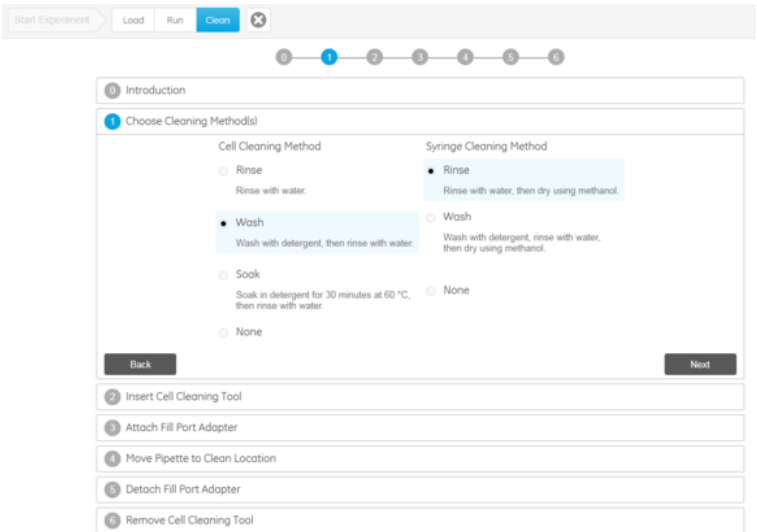
Follow the experiment run in real-time. The instrument status is displayed in the right live pane.

Clean the instrument

Clean workspace

This workspace is found in the **Run Experiment** view. Follow the guided workflow that steps the user through cleaning both the sample cell and the titration syringe. Additionally, the user has the option of watching videos detailing the procedure.

Step	Action
1	Click **Clean** *Result:* The Clean workspace opens. 
2	To view an instruction video showing all the default cleaning steps, click the **Play** button in the instruction video window.
3	To start the clean instrument workflow click **Next**. Select what cleaning methods to use. In most cases you can use the default cleaning methods.

Step	Action
	

Note: Software-instrument connection is required to clean the instrument.

6 MAINTENANCE

This section provides the user with basic information on the proper maintenance of the instrument. For a more detailed description of maintenance procedures, watch the videos provided with the software (in English only).

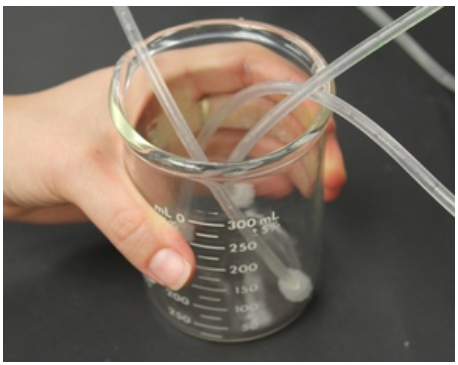
The following topics are covered:

Washing module

Remove bottles

Use this procedure to remove the bottles for cleaning. The lines to the bottles need to be evacuated before the tubing is disconnected from the wash station. This will make sure no solution leaks from the disconnected tubing.

Step	Action
1	Disconnect the tubing from the bottle while leaving the other end attached to the system and place the disconnected ends in a clean, empty beaker.

Step	Action
	
2	Start an instrument cleaning cycle using only the rinse cell cleaning method (no syringe clean). Repeat if the instrument faults due to absence of fluid. Repeat using only the rinse syringe cleaning method (no cell clean). See Clean the instrument
3	Remove the tubing from the bottle(s) by twisting the Luer lock fitting counterclockwise.
4	Fill the bottle(s).
5	Secure the bottle(s).
6	Install the tubing to the bottles by twisting the Luer lock fitting clockwise. **Note**: Do not overtighten.

Prime the tubing

Prime the tubing from the bottles to the washing module to make sure that the full volume of cleaning fluid is delivered. This procedure is required only if the tubing from the bottles to the washing module have been drained of fluid and contain air.

Step	Action
1	Make sure all bottles contain enough cleaning fluid and all fluid lines are connected.
2	Start an instrument cleaning cycle using the rinse cell and rinse syringe cleaning methods. See Clean the instrument.

When the tubing has been primed (visibly clear of air bubbles), the system uses the majority of the remaining procedure time to dry the syringe. This occurs with approximately eight minutes remaining in the procedure. You can let the procedure finish or click **Cancel** at this time.

Replace the bottle filters

Follow this procedure if the volume delivery has decreased due to a clogged filter. Follow this procedure for one bottle at a time.

Step	Action
1	Disconnect the tubing from the top of the bottle.
2	Unscrew the bottle cap.
3	Remove the filter at the end of the tubing by gently pulling the filter from the tube.
4	Install a new filter at the end of the tubing.
5	Replace the bottle cap.
6	Reconnect the tubing to the bottle cap.

Empty the waste bottle

Follow this procedure to empty the waste bottle.

Step	Action
1	Verify that the system is in an idle state.
2	Unscrew the grey cap of the waste bottle by turning the lid counter-clockwise.
3	Empty the bottle according to your laboratory waste handling procedures.
4	Reattach the cap by turning it clockwise until it is snug.

Note: If the waste bottle is full, the excess will fill the overflow bottle and shut down the vacuum pump.

Replace the syringe plunger tip

Introduction

The plunger tip forms a seal with the syringe glass. The tip spins along with the syringe glass while the metal plunger itself remains stationary. As the plunger drives titrant out of the syringe glass, plunger tip wear can occur. Too much wear can result in poor data. If left unreplaced, the metal plunger can break through the worn plunger tip.

Replace the plunger tip every 300 experiments or at the first sign of wear.

To replace the syringe plunger tip, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

Note: Instrument connection is required to replace the syringe plunger tip.

The Change Plunger Tip workflow

The software guided The Change Plunger Tip workflow is divided into the following steps:

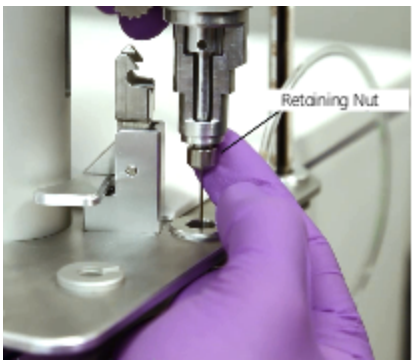
Stage	Description
1	Move Pipette to Clean Location
2	Move Pipette to Load Location
3	Remove Syringe Glass and Plunger Tip
4	Install Plunger Tip
5	Install Syringe Glass

Move the pipette to the Clean Location

To move the pipette to the Clean Location, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

Step	Action
1	Move the pipette partway into the Clean Location.
2	Grasp the rotating assembly of the pipette with one hand, and remove the steel retain-

Step	Action
	ing nut where the paddle stem meets the pipette with your other hand. 
3	Let the retaining nut drop into the Clean Location.

Move the pipette to the Load Location

To move the pipette to the Load Location, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

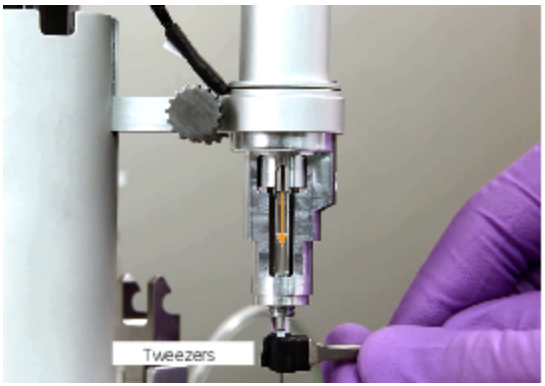
The software guided workflow is also shown in the following table.

Step	Action
1	Make sure that the Load Location does not contain a microcentrifuge tube.
2	Move the pipette to the Load Location. **Note**: It should not be necessary, but have your hand ready to receive the syringe glass.
3	Click **Next**. *Result*: The instrument will move the plunger down and press out the syringe glass.

Remove the syringe glass and the plunger tip

To remove syringe glass and plunger tip, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

Step	Action
	Carefully remove the syringe glass using the supplied tweezers.
	
1	If the plunger motion did not expose enough syringe glass, use the Syringe Glass Removal Tool.
	
2	Insert the Plunger Tip Removal Tool into the empty pipette housing.

Step	Action
	
3	Apply pressure until the tool slides over the tip.
4	Remove the tool.
5	Make sure that the tip was removed.

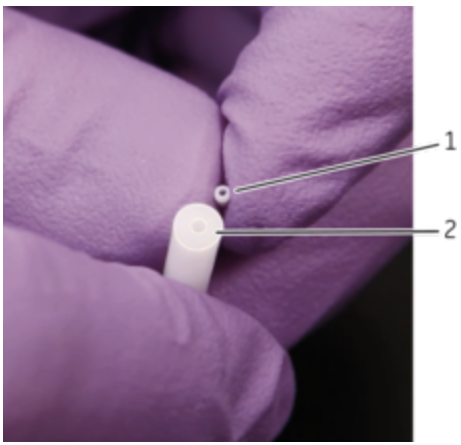
Install the plunger tip

To install the plunger tip, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

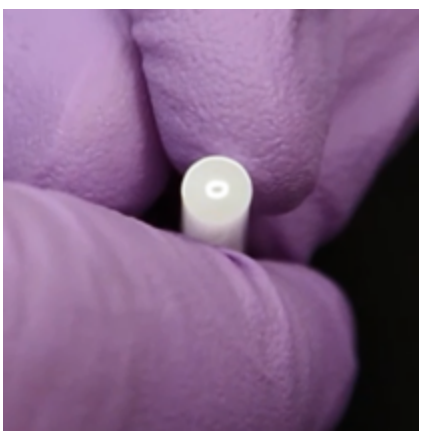
Step	Action
1	Install a new plunger tip in the Plunger Tip Install Tool. Pay careful attention to the orientation of the plunger tip.

Step	Action



Part	Function
1	Plunger tip
2	Plunger Tip Install Tool

The plunger tip opening should face out from the tool.



2	Insert the tool into the pipette housing. You will meet resistance as the new plastic tip slides over the plunger. A very soft click

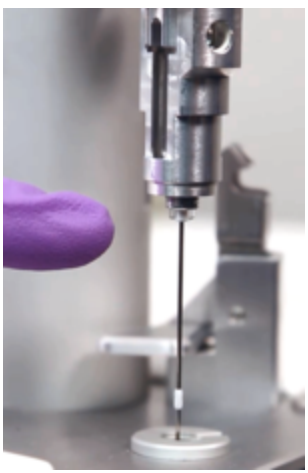
Step	Action
	should be felt when it is seated.
	
	Note: Do not apply any additional pressure.
3	Remove the tool.

Install the syringe glass

To install the syringe glass, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

Step	Action
1	Grasp the pipette's rotating assembly.
2	Gently insert the syringe glass with the syringe's fill port facing the same direction as the hole in the pipette's rotating assembly.

Step	Action
	
	If aligned properly, the installed syringe glass should leave approximately 2 mm of glass exposed.
	
3	Leave the retaining nut in the Clean Location, and insert the pipette into the Clean Location without fully engaging the clamp.
4	Start to reinstall the retaining nut by turning the pipette's rotating assembly with its threads engaged with the retaining nut.
5	Move the pipette to the Rest Location and finish reinstalling the retaining nut.

Replace the titration syringe

Introduction

The syringe can be replaced as a part of preventive maintenance.

A broken syringe will not operate properly, will likely result in poor experimental results, and could contaminate the cell with broken glass.

WARNING! Hazardous substances and biological agents. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of MicroCal PEAQ-ITC.

To replace the titration syringe, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following tables.

Note: Instrument connection is required to replace the syringe plunger tip.

The Replace Syringe workflow

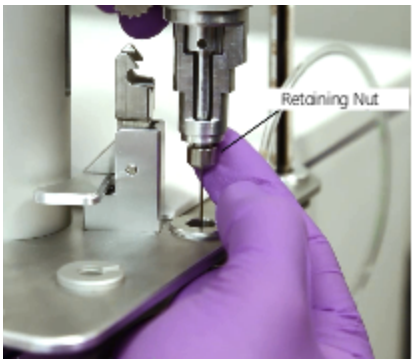
The software guided Replace Syringe workflow is divided into the following steps:

Stage	Description
1	Move Pipette to Clean Location
2	Move Pipette to Load Location
3	Remove Syringe Glass
4	Install Syringe Glass

Move the pipette to the Clean Location

To move the pipette to the Clean Location, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

Step	Action
1	Move the pipette partway into the Clean Location.
2	Grasp the rotating assembly of the pipette with one hand, and remove the steel retaining nut where the paddle stem meets the pipette with your other hand. 
3	Let the retaining nut drop into the Clean Location.

Move the pipette to the Load Location

To move the pipette to the Load Location, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

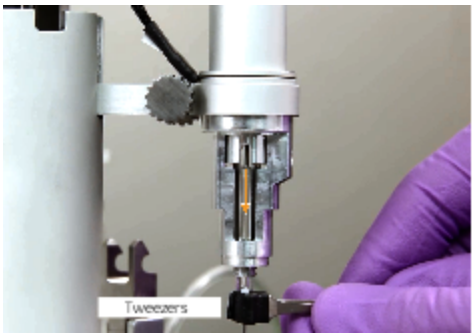
The software guided workflow is also shown in the following table.

Step	Action
1	Make sure that the Load Location does not contain a microcentrifuge tube.
2	Move the pipette to the Load Location. **Note**: It should not be necessary, but have your hand ready to receive the syringe glass.
3	Click **Next**. *Result*: The instrument will move the plunger down and press out the syringe glass.

Remove the syringe glass

To remove the syringe glass, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

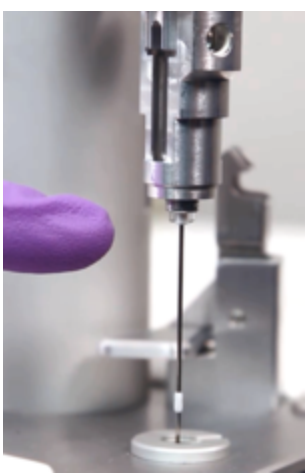
The software guided workflow is also shown in the following table.

Step	Action
1	Carefully remove the syringe glass using the supplied tweezers. 
2	Use the syringe glass removal tool if the plunger motion did not expose enough syringe glass. 

Install the syringe glass

To install the syringe glass, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

Step	Action
1	Grasp the pipette's rotating assembly.
2	Gently insert the syringe glass with the syringe's fill port facing the same direction as the hole in the pipette's rotating assembly.  If aligned properly, the installed syringe glass should leave approximately 2 mm of glass exposed. 
3	Leave the retaining nut in the Clean Location, and insert the pipette into the Clean Location without fully engaging the clamp.
4	Start to reinstall the retaining nut by turning the pipette's rotating assembly with its threads engaged with the retaining nut.
5	Move the pipette to the Rest Location and finish reinstalling the retaining nut.

Clean the titration syringe

Introduction

A dirty syringe can result in poor data.

Detergent cleaning of the syringe between runs is recommended if performing reverse titrations (protein is loaded into the syringe). If poor data persists after extensive cell cleaning, remove the syringe for cleaning.

WARNING! Hazardous substances and biological agents. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of MicroCal PEAQ-ITC.

To clean the titration syringe, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following tables.

Note: Instrument connection is required to replace the syringe plunger tip.

The Clean Syringe workflow

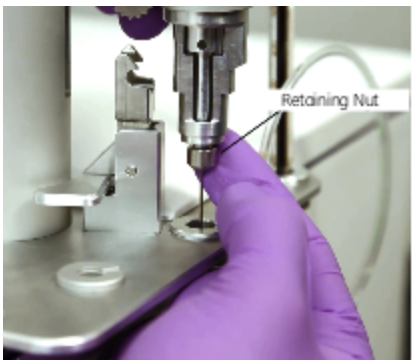
The software guided Clean Syringe workflow is divided into the following steps:

Stage	Description
1	Move Pipette to Clean Location
2	Move Pipette to Load Location
3	Remove Syringe Glass
4	Clean Syringe Glass
5	Install Syringe Glass

Move the pipette to the Clean Location

To move the pipette to the Clean Location, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

Step	Action
1	Move the pipette partway into the Clean Location.
2	Grasp the rotating assembly of the pipette with one hand, and remove the steel retaining nut where the paddle stem meets the pipette with your other hand. 
3	Let the retaining nut drop into the Clean Location.

Move the pipette to the Load Location

To move the pipette to the Load Location, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

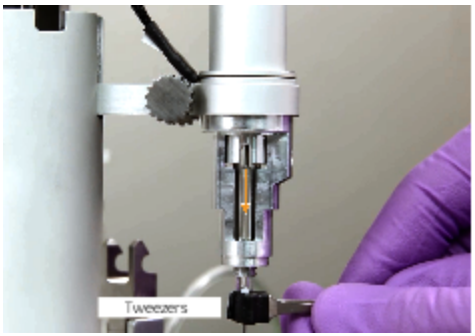
The software guided workflow is also shown in the following table.

Step	Action
1	Make sure that the Load Location does not contain a microcentrifuge tube.
2	Move the pipette to the Load Location. **Note**: It should not be necessary, but have your hand ready to receive the syringe glass.
3	Click **Next**. *Result*: The instrument will move the plunger down and press out the syringe glass.

Remove the syringe glass

To remove the syringe glass, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

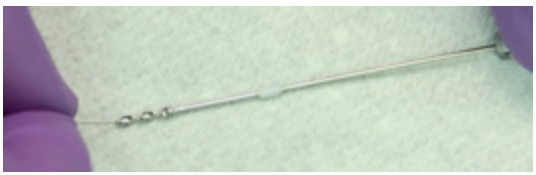
Step	Action
1	Carefully remove the syringe glass using the supplied tweezers. 
2	Use the syringe glass removal tool if the plunger motion did not expose enough syringe glass. 

Clean the syringe glass

Clean the glass syringe occasionally by hand, as the instrument does not clean the entire inner diameter of the glass syringe. If there is any blockages in the syringe, these should be taken care of before cleaning using the supplied Cleaning Wire.

To remove any blockages in the paddle stem and clean the inner diameter of the glass syringe, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

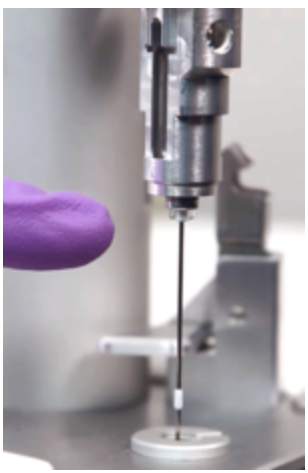
Step	Action
1	Carefully insert the supplied Cleaning Wire into the end of the paddle and pass it up through the stem into the syringe glass. 
2	Scrub the inside of the syringe with detergent using the supplied Syringe Glass Cleaning Brush. 
3	Rinse thoroughly with water to remove as much detergent as possible.
4	Dry the syringe to prevent sample dilution.

Install the syringe glass

To install the syringe glass, follow the instruction video and/or the software guided workflow integrated in the MicroCal PEAQ-ITC Control Software.

The software guided workflow is also shown in the following table.

Step	Action
1	Grasp the pipette's rotating assembly.
2	Gently insert the syringe glass with the syringe's fill port facing the same direction as the hole in the pipette's rotating assembly.

Step	Action
	
	If aligned properly, the installed syringe glass should leave approximately 2 mm of glass exposed.
	
3	Leave the retaining nut in the Clean Location, and insert the pipette into the Clean Location without fully engaging the clamp.
4	Start to reinstall the retaining nut by turning the pipette's rotating assembly with its threads engaged with the retaining nut.
5	Move the pipette to the Rest Location and finish reinstalling the retaining nut.

Refill the reference cell

Introduction

The MicroCal PEAQ-ITC instrument has two cells, the sample cell and the reference cell. The reference cell must be refilled manually. An underfilled reference cell can result in a starting baseline position *greater* than specified in the ITC Method.

Refilling the reference cell is recommended approximately once a week.

Procedure

The following steps describes how to refill the reference cell.

Step	Action
1	Gently insert a glass Hamilton syringe into the reference cell until it touches the bottom.
2	Empty the reference cell completely by pulling up the syringe plunger. **Note**: Make sure that no bubbles are trapped in the cell.
3	Remove and empty the syringe. Clean the syringe, if necessary.
4	Pull approximately 300 µl of degassed, distilled water into the syringe. Tap the syringe glass gently so that all the bubbles are at the top volume of the syringe.
5	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 (as illustrated in the transparent cell model), and hold it there during the filling process.

Step	Action
	
6	Inject the water solution slowly into the cell until it spills out over the top of the cell stem. Dislodge any trapped bubbles with several abrupt spurts of the water solution. **Note**: Make sure no bubbles are transported into the reference cell while loading the water solution.
7	Lift the tip of the syringe to the cell port (just below the visible portion of the cell port) and remove the excess water solution.
8	Remove the syringe. Install the reference cell cover to prevent evaporation.

7

EXPERIMENTAL DESIGN

This section provides guidelines on how to design an ITC experiment. The Design Experiment workspace can be used to create and simulate experiments with alternative method parameter settings than the preinstalled default method parameter settings for the different fitting models in the MicroCal PEAQ-ITC Analysis Software.

The following topics are covered:

Guided experimental design

Introduction

The **Guided** mode of the **Experimental design** view helps you design one-to-one binding experiments and simulate basic runs.

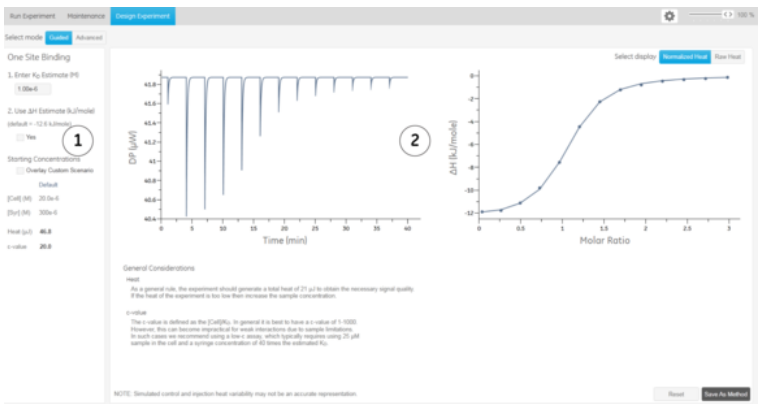
- Click the **Guided** tab to enter Guided mode.

Guided mode workspace

The workspace is divided into two panes.

In the left, design pane you can enter experiment estimates and read the recommended concentrations for the current estimates.

The right, simulation pane displays two graphs showing simulated raw injection data and integrated injection data.



Pane	Function
	Design pane
1 (left pane)	In this pane you can: • enter estimates for the dissociation constant (K_D) and

Pane	Function
	heat change (ΔH) • read the recommended concentrations for the current estimates.
2 (right pane)	**Simulation pane** This pane displays the simulation by two graphs showing: • the raw injection data, and • the integrated injection data. In this pane you can also read a discussion on how to design the experiment using the currently set estimates.

Enter an estimate of the disassociation constant

In the left pane, enter an estimation of the dissociation constant for the ligand-protein system used in the experiment in the **Enter K_D estimate (M)** box.

Enter an estimate of heat exchange

If you want to enter an estimate of heat change for the ligand-protein system:

Step	Action
1	In the left pane, select the **Yes** check box.
2	Enter the estimate in the **Use ΔH Estimate (kcal/mole)** box.

Simulate custom concentrations

If you want to simulate custom concentrations:

Step	Action
1	In the left pane, select the **Overlay Custom Scenario** check box.
2	Enter the concentrations in the **[Cell] (M) and [Syr] (M)** boxes. Result: The simulated runs are displayed in the right pane.

Starting concentrations

The starting concentrations for the currently set estimates are displayed below the heading Starting Concentrations in the left pane.

Default values for the concentration in the cell, the concentration in the syringe, the total heat of the experiment, and the c-value are shown, and can be adjusted by selecting the **Overlay Custom Scenario**.

If a less than ideal experiment is simulated, the **Heat** and **c-value** can be accompanied by warnings.

Simulated graphs

The right pane displays simulations of raw injection data in the left chart and a fitted curve of the simulated integrated injections data in the right chart.

There are two display options for the simulated integrated injections data plot, normalized heat and raw heat.

Reset button

Click **Reset** to reset all simulation and experimental parameters.

Save as Method button

You can save simulation and experimental parameters as method files. Save as the **ITC Method** file type for methods that are to be used in MicroCal PEAQ-ITC. Save as the **INJ** file type for methods that are to be used in MicroCal PEAQ-ITC Automated.

Step	Action
1	Click **Save as Method**. *Result:* The **Save As** dialog opens.
2	Type a file name in the **File name** box if necessary.
3	Select file type in the **Save as type** list.
4	Browse to select a destination to save the file.
5	Click **Save**. *Result*: The method will be saved in the file format selected above.

Advanced experimental design

Introduction

Advanced experimental design can be performed in the **Advanced** mode of the **Design Experiment** view.

It is possible to edit more experimental parameters in the Advanced mode than in the **Guided** mode, but you will not receive recommendations or comments as help text.

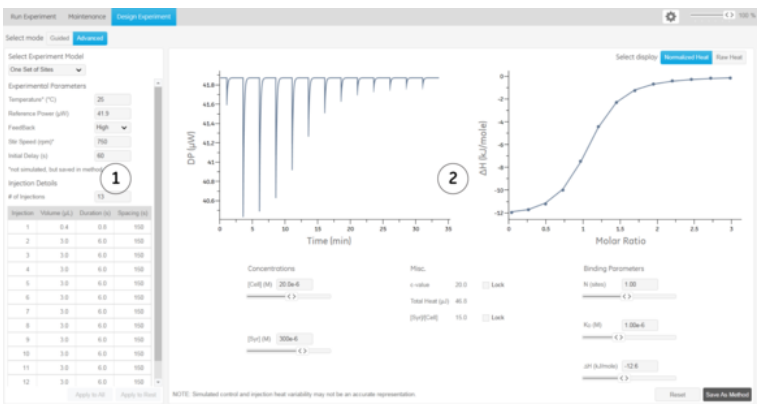
- Click the **Advanced** tab to enter Advanced mode.

Advanced mode workspace

The workspace is divided into two panes.

Select fitting model and experimental parameters in the left, design pane.

The right, simulation pane displays two graphs showing simulated raw injection data and integrated injection data.



Pane	Function
1 (left pane)	**Design pane** In this pane you can:

Pane	Function
	• select fitting model • edit the experimental parameters for the instrument • edit injection details.
2 (right pane)	**Simulation pane** This pane displays the simulation by two graphs showing: • the raw injection data, and • the integrated injection data. In this pane you can also adjust the experimental parameters for the simulation.

Experimental method

You can select fitting model for your simulation in the Select Experiment Method menu in the left, design pane.

The selectable models are:

- One Set of Sites
- Competitive Binding
- Two Sets of Sites
- Dissociation
- Sequential Binding Sites

Instrument settings

You can edit the settings for the instrument in the left, design pane.

Note: Not all settings reflect a change in the simulation, but they will be reflected in a saved method file.

Instrument setting	Description
Temp (°C)	Enter the desired run temperature for the experiment. The instrument's operating range is 2°C to 80°C. Most runs

<table>
<tr><th>Instrument setting</th><th>Description</th></tr>
<tr><td></td><td>are performed at 25°C (room temperature).</td></tr>
<tr><td>Reference Power</td><td>Enter a value for reference power. The differential power (DP) baseline will equilibrate near this value.

The reference power is a small constant amount of power supplied to the offset heater of the reference cell. This causes the DP feedback system to supply compensating power to the sample cell to equilibrate the temperatures. The best choice for the reference power setting can be determined by the anticipated size and sign of the titration heats. The following table gives some guidelines.

<table><tr><th>Expected reaction type</th><th>Suggested reference power</th></tr><tr><td>Large exothermic</td><td>Large value (approx. 10 µcal/s using high feedback)</td></tr><tr><td>Large endothermic</td><td>Small value (approx. 0.5 µcal/s)</td></tr><tr><td>Unknown</td><td>Intermediate value (5 µcal/s using high feedback)</td></tr></table></td></tr>
<tr><td>FeedBack</td><td>The feedback mode affects both response time and sensitivity. High gain provides the fastest response time. No gain (passive mode, None) provides the highest sensitivity.

Most ITC reactions require using the High setting.

Monitoring long, slow thermal processes (for example, kinetics, metabolic rates) might benefit from using the None (passive) or Low settings.</td></tr>
<tr><td>Stir speed (rpm)</td><td>Enter the sample cell stirring speed in revolutions per minute. Faster stirring may be necessary if the sample cell contains suspended particles, for example agarose beads.</td></tr>
<tr><td>Initial Delay (s)</td><td>Enter the time (s) between the start of the run and the first injection (standard value 60 s). This is necessary to establish a baseline before the first injection.</td></tr>
</table>

Injection settings

You can edit the injection settings in the left, design pane.

Injection setting	Description
# of Injections	Enter the number of injections for the titration (ITC) experiment. The multiple injection method requires a minimum of 10 to 15 injections. The single injection method uses one injection.
Volume(µL)	Enter the volume (µl) of titrant to be injected from the pipette into the sample cell for the injection(s) selected in the injection list.
Duration(s)	Enter how long (seconds) each injection of titrant into the sample cell for will be.
Spacing(s)	Enter the time (seconds) between the beginning of the injection(s) selected in the injection list and the beginning of the next injection (or end of run). The injection spacing must allow enough time between injections to allow the DP signal to return to the baseline after an injection peak deflection. Typical values for this parameter range from 90 to 180 seconds, depending on the feedback mode, temperature and reaction kinetics. **Note**: For the single injection method, the spacing should be at least 90 seconds longer than the duration of the injection.

Edit injections

- Double-click a box in the injection list to edit an injection.
- Click **Apply to all** to copy the selected injection's settings to all other injections in the list.
- Click **Apply to Rest** to copy the selected injection's settings to all following injections in the list.

Simulated graphs

In the right, simulation pane, you can follow your simulation and adjust simulations parameter, such as concentration and binding parameters.

The upper section displays two graphs that are used to evaluate the simulation, one displaying raw injection data and one displaying integrated injection data.

There are two display options for the integrated injection data plot, normalized heat and raw heat.

The lower section provides options for adjusting the simulation parameters.

Note: All graphs and simulation parameters displayed in this pane are specific for the fitting model chosen.

Reset button

Click **Reset** to reset all simulation and experimental parameters.

Save as Method button

You can save simulation and experimental parameters as method files. Save as the ITC Method file type for methods that are to be used in MicroCal PEAQ-ITC. Save as the INJ file type for methods that are to be used in MicroCal PEAQ-ITC Automated.

Step	Action
1	Click **Save as Method**. *Result:* The **Save As** dialog opens.
2	Type a file name in the **File name** box if necessary.
3	Select file type in the **Save as type** list.
4	Browse to select a destination to save the file.
5	Click **Save**. *Result*: The method will be saved in the file format selected above.

8

TROUBLESHOOTING

This section provides information on how to solve problems that may arise.

The following topics are covered:

Troubleshooting overview

Contact information

Please contact Malvern Instruments for any instrument or data analysis questions or issues you may have.

Include data file

When emailing 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. Where the problem or question relates to post run data analysis, it is best to attach the raw data file (*.itc).

Diagnosing the problem

Perform the following minimum diagnostic steps prior to requesting service:

Step	Action
1	Run a thorough cleaning routine with detergent.
2	Load both the cell and syringe with degassed distilled water.
3	Run a water into water titration with at least 15 injections of 2µl each.

If, after completion of the steps listed above, the MicroCal PEAQ-ITC performance is not corrected, please contact the Malvern Instruments help desk. The water runs should be provided to the Malvern Instruments technician for evaluation. Following evaluation, a representative will contact you with comments and recommendations.

Troubleshooting chart

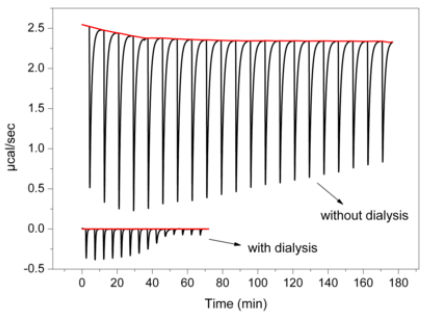
Error symptom	Corrective action
Instrument not running	• Check that power is plugged in and switched on. • Check that the USB cable is properly connected. • Check that MicroCal PEAQ-ITC Control Software is running and properly initialized.
Software reports networking errors	Unplug the network port from controller PC, reboot the Controller PC, and restart the control software.
Instrument not working properly	• Visually check that the syringe is straight, that no fluids are leaking and that the 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.
Control software reports initialization errors, communication problems, or hardware errors not covered in this manual	Contact your Malvern Instruments service representative.
Result data shows artifacts	• Check that the reagent bottles are not empty and are properly attached to the correct ports. • Refill the reference cell (see Refill the reference cell). If these steps do not resolve the problem, see How to get help.
Titration syringe is damaged	See Replace the titration syringe for replacement.

Peaks are too large

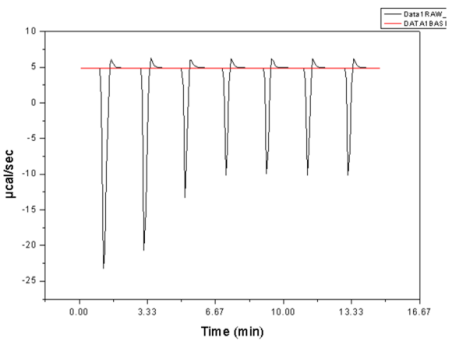
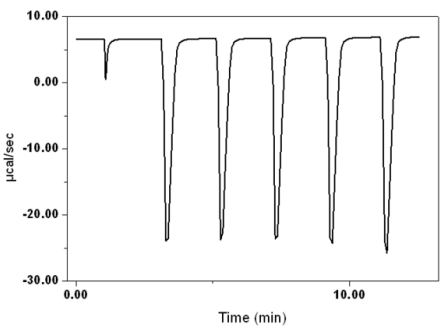
Introduction

Baselines should always be within 1 °cal/s of the user-specified reference power. Normal baseline noise is visible between the tiny water-into-water injection peaks if the titration syringe and paddle stem are properly cleaned and completely dry.

Buffer mismatches are often the cause of peaks being too large. Buffer exchange, for example through dialysis, is often a solution. The following figure shows a binding event before and after dialysis.



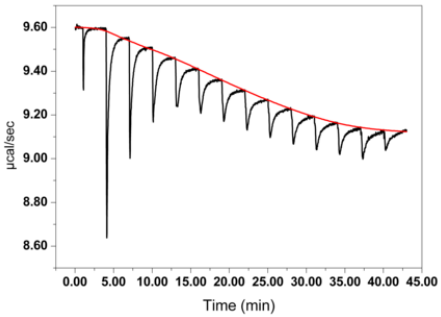
Problem causes

Cause	Corrective action
A buffer mismatch between the titrant and cell material.	See Preparing the samples.
Methanol: All samples have consistently large peaks.   	The peaks will exhibit extreme heat signatures in the presence of any residual methanol. The "bounce" after each injection is typical when the signal goes below 0. • Check wash module fittings. • Make sure that the syringe is being adequately dried.

Broad peaks

Introduction

To measure the heat accurately, the spacing between injections should be sufficient to allow the signal to return to baseline.



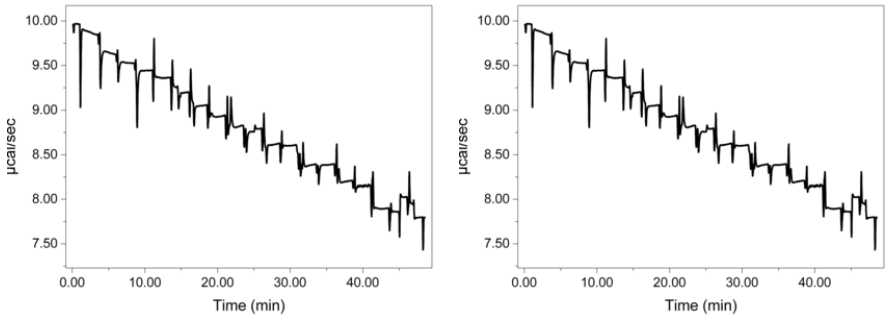
Problem causes

Cause	Corrective action
Injection spacing is too short	Increase the injection spacing in the method file or change the injection spacing while the instrument is running. See Advanced experimental design.
Feedback mode is set to an unexpected value. This directly affects the response time of the instrument. Low feedback (or none) requires larger injection spacing than the high feedback setting.	Check the feedback setting and adjust it, or the injection spacing, accordingly.
The kinetics of the system can also affect the time required to return to baseline. If a given system routinely takes a long time, and the injection spacing is set to just return to baseline, on rare occasions the baseline fitting algorithm will not perform well.	Increase injection spacing.

Downward stepping baseline

Introduction

The baseline might start in the normal range, within 1 °cal/s of the reference power, but after each injection, the baseline steps down. The heat capacitance of the sample cell also decreases with each injection.



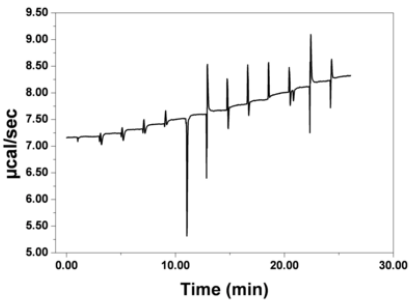
Problem causes

Cause	Corrective action
The titration syringe is empty or underfilled.	The syringe injects air into the cell, which shifts the heat capacity of the sample cell and offsets the baseline.
The small fill port adaptor tip that fits into the fill port in the syringe is damaged.	Contact a Malvern Instruments representative.

Upward stepping baseline

Introduction

The upward steps result from the sample cell getting more full with each injection. The heat capacitance of the sample cell also increases with each injection.



Problem causes

Cause	Corrective action
The sample cell is dirty.	Clean the sample cell.
The sample cell is underfilled.	Make sure the cell was loaded with enough sample (minimum 280 µl).

Reversed/oscillating peaks

Introduction

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

Problem causes

Cause	Corrective action
Reversed/oscillating peaks	Set the reference power higher.

Cause	Corrective action
This oscillatory behavior is due to the differential power dropping below 0.	

Baseline spikes

Introduction

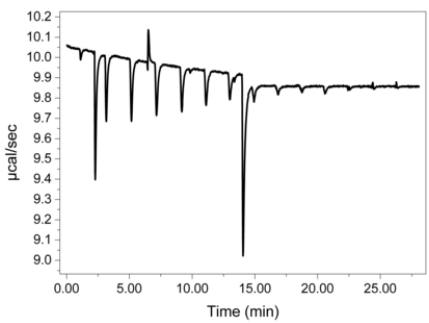
If there are spikes in the baseline, the automated baseline fitting may not function properly.

There are two types of bubble spikes, both types are caused by gas bubbles coming out of solution when the temperature of the sample solution is increased to reach experimental temperature.

- Sharp, isolated spikes.
- Prolonged noise spikes which occur more often as the experimental temperature increases.

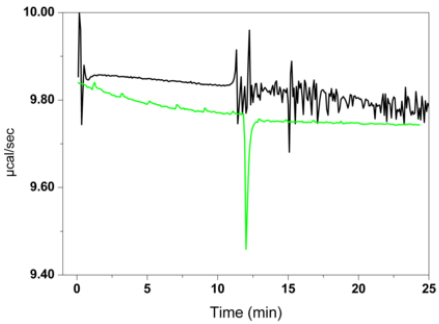
Sharp spikes

The following graph displays sharp isolated baseline spikes.



Prolonged noise spikes

The following graph exhibits prolonged noise baseline spikes (in black), as well as a sharp, isolated spike (in green).



Problem causes

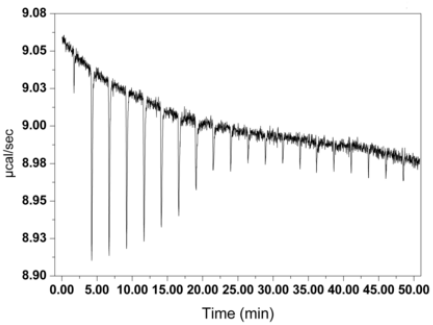
Cause	Corrective action
Air bubbles are trapped in the cell.	• Degas the sample solution properly (see Preparing the samples). • Spikes are convoluted with an injection. Discard that data point and manually fit using MicroCal PEAQ-ITC Analysis Software. • Spikes are confined to an injection's baseline. Save the data point by removing the spike in MicroCal PEAQ-ITC Analysis Software.

Low baseline

Introduction

If the baseline settles at more than 1 °cal/s below the user-specified reference power, the results may be less than optimal. For example, the reference power was set to 10 in the example illustrated below. The data looks fine, aside from the displaced baseline position.

However, the stoichiometric result may be slightly affected.



Problem causes

Problems with a low baseline are often caused by an underfilled sample cell or reference cell.

Cause	Corrective action
Dirty cell caused a poor load.	Clean the sample cell.
Air bubbles are trapped in the cell.	Degas the sample solution properly.
The sample cell is underfilled.	Make sure the cell was loaded with enough sample (minimum 280 µl).
A baseline position larger than the reference power due to an underfilled (or evaporated) reference cell.	Fill the reference cell (see Refill the reference cell)

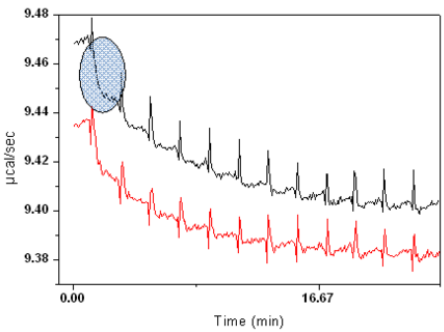
Abnormal peaks

Introduction

A few examples of abnormal peaks are illustrated in the following examples.

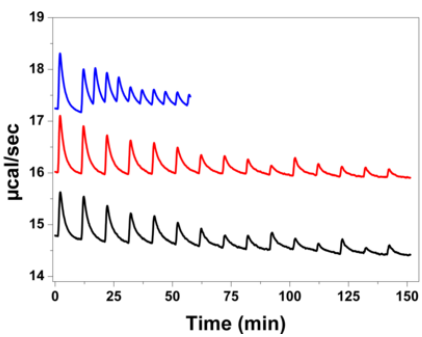
Example 1

The repeating trend displayed in the following illustration implies that the sample cell needs cleaning.



Example 2

In the following illustration, there was not enough time between injections. Increase the spacing between injection and/or check the feedback settings. See Broad peaks for a similar discussion.



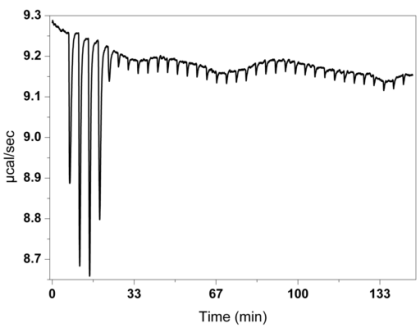
Unexpected thermodynamic results

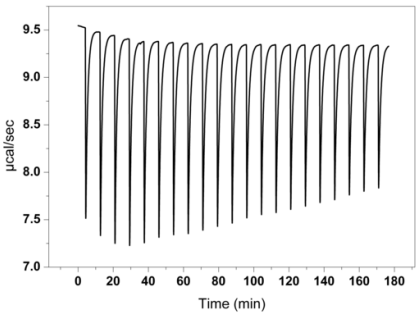
Introduction

Often results do not yield a "textbook" sigmoidal binding isotherm. This may be a result of the system itself, or sample preparation, or both. Several scenarios are described in the following topics to help diagnose the problem.

Problem causes

There are several causes of unexpected thermodynamic results. Keep the following scenarios in mind when troubleshooting results:

Observed result	Corrective action
Stoichiometry (n) varies with enthalpy (ΔH).	Check that the syringe concentration is correct.
Stoichiometry (n) varies alone.	Check that the cell concentration is correct.
Early saturation.	• Increase protein concentration or decrease ligand concentration. • Examine sample preparation (see Preparing the samples). 
Experimental heats are same as control heats.	Change experimental temperature by at least 10°C and/or increase sample concentration.
No saturation.	Weaker-than-expected binding or buffer mismatch.

Observed result	Corrective action
	

Washing Module

Washing module troubleshooting is described in the following topics.

No flow during washing

Cause	Corrective action
Tubing connection	Make sure that all connections are tight
Clogged pump	Call for Service
Clogged bottle filter	See Replace the bottle filters
Vacuum tube not tight	Tighten the vacuum tubing
Reagent bottles are empty	Refill reagent bottles

Syringe fill failure

Cause	Corrective action
Tubing connection	Make sure that all connections are tight
Clogged syringe paddle	See Replace the titration syringe on page 77 for instructions on how to clean the titration syringe.
Damaged Filling Port Adapter	Check that the tip is not deformed
Broken glass	1. Make sure that the syringe glass near the fill port has not been damaged 2. Call for Service

Syringe not dry

Cause	Corrective action
Methanol bottle empty	Fill the methanol bottle

Washing module not recognized by MicroCal PEAQ-ITC during hardware detection

Cause	Corrective action
USB connection problem	Verify that all USB connections are tight at the hub and controller PC

Washing module pump does not run

Cause	Corrective action
Power connection problem	Verify that the coaxial power connector is securely connected to the rear of the washing module

9

REFERENCE

This section provides reference information that may be useful when installing, operating, maintaining and troubleshooting the MicroCal PEAQ-ITC system.

The following topics are covered in this section:

MicroCal PEAQ-ITC specifications

Physical specifications

Property	Value
Cell material	Hastelloy Alloy C-276
Weight:	
Fully assembled	13.6 kg
Dimensions:	
Fully assembled (W x H x D)	43 × 38 × 46 cm

Electrical specifications

Property	Function
Electrical ratings:	
Voltage	100-240 VAC (power adapter), 24 VDC (power supply to the instrument)
Frequency	50/60 Hz
Power	150 W
Output	Secondary/Data connection only
Mode of operation	Continuous
Classification	Class I

Site requirements

Property	Function
Bench space and load	• width ≥ 120 cm • depth ≥ 64 cm with Controller PC depth ≥ 51 cm without Controller PC • free height above bench ≥ 80 cm

Property	Function
	• rated for at least 115 kg
Clearance	• ≥ 15 cm behind the instrument • ≥ 40 cm in front of the instrument Service functions will require an additional 30 cm overhead clearance.

Reagent requirements

Introduction

The MicroCal PEAQ-ITC requires the following cleaning solutions.

Recommended reagents

- Distilled water
- Recommended detergents are 20% Contrad™ 70 or 14% Decon™ 90.

  Contrad™ 70 and Decon™ 90 contain dodecylbenzensulfonic acid, potassium hydroxide, sodium citrate and sodium laurel ether sulfate. It is biodegradable and can easily be rinsed off.
- ≥ 99% pure methanol ("HPLC Grade" is recommended)

Warning! Methanol is highly volatile and can be hazardous to humans.

- Storage containers should be kept tightly closed.
- Methanol should always be transferred in a well-ventilated area with no ignition sources. The operator should have protective clothing, eye protection and 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.

Chemical resistance guide

Introduction

This section specifies the chemical resistance of MicroCal PEAQ-ITC to some of the most commonly used chemicals in isothermal titration calorimetry.

List of tested compatible chemicals

Note: A user can be exposed to large volumes of chemical substances over a long time period. Material Safety Data Sheets (MSDS) provide the user with information regarding characteristics, human and environmental risks and preventive measures. Make sure that you have the MSDS available from your chemical distributor and/or databases on the internet.

Chemical	Concentration
Potassium acetate pH 5.5	100 mM
Sodium citrate pH 4.0	100 mM
Glycine pH 3 and pH 10	100 mM
PBS buffer pH 7.4	100 mM
HEPES pH 7.0	100 mM
MES pH 5.6	100 mM
NaCl	1 M
Dithioerytritol (DTE)	20 mM
DMSO	50%
Acetonitrile	50%
Methanol	100%
Ethylene Glycol	50%
Contrad 70	20%
Decon 90	14%

Wetted materials

Introduction

This section specifies the wetted materials of MicroCal PEAQ-ITC.

Wetted surface materials

The following wetted surface materials are used in MicroCal PEAQ-ITC:

- Hastelloy C276
- 316 SST
- PEEK
- Kalrez™
- PTFE
- Borosilicate glass
- EPDM
- ULTEM™
- Tygon™ 2375
- ETFE
- Aluminium oxide

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Malvern Instruments Limited
Grovewood Road, Malvern
Worcestershire, WR14 1XZ, UK

Tel +44 1684 892456
Fax +44 1684 892789

www.malvern.com

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