



**Malvern
Panalytical**
a spectris company

MICROCAL PEAQ-DSC USER GUIDE



MICROCAL PEAQ-DSC USER GUIDE

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CHAPTER 1 INTRODUCTION

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About this manual

This manual provides the essential information necessary for ensuring safe and efficient operation of the MicroCal PEAQ-DSC system. This manual provides:

- Information about the system's security features
- Instructions for configuring the system settings
- Instructions for operating the system, creating and running a sequence, analyzing the results, and producing reports
- Reference information for advanced users

Product documentation structure

This manual fits into the following information structure for this product:

- **Basic Guide** - the essentials required to get started, including health and safety. All users must read this manual before using the system.
- **User Guide** - provides detailed information on how to use and administer the system.
- **Help system** - integrated with the system software, provides information on all software features.



WARNING!

The instrument and the samples measured may be hazardous if misused. Users must read the Health and Safety information in the Basic Guide before operating the system.

Scope of this document

This manual covers the **MicroCal PEAQ-DSC** system which does not include the Autosampler. Samples are loaded manually.

Naming convention

The MicroCal PEAQ-DSC system is referred to either as the MicroCal PEAQ-DSC, or as *the instrument*. The combination of the instrument and the computer is referred to as *the system*.

Menu items

Menu items and screen elements in the software are shown in **bold** text. Commands prompting you to select an option or item are also shown in **bold** text.

For example, the command *Click the **Experiment** page* refers to selecting the page titled **Experiment** in the software. The command *Click the **Open** button* refers to clicking the mouse on the **Open** button.


Where to get help

This section gives information on the various channels in place to get help with your MicroCal PEAQ-DSC system.

Website



Our website offers users a comprehensive range of resources for use by customers. It gives free access to exclusive content including webinars, presentations, application notes, technical notes, whitepapers, software downloads and more. Visit www.malvernpanalytical.com.

Help system

A full help system is supplied with your Malvern Panalytical software system. This provides detailed reference information on all software features. To access this, click the **Help**  icon located in the upper right corner of the main screen.

Help desk

All queries about the system should be directed to your local Malvern Panalytical representative, providing the following information:

- **Model and serial number** of the instrument. It can be found on the rear panel or casing of the instrument and/or by navigating to the **Application**  menu, then **Options** in the software and viewing the license.
- **Software version** (see the **Application**  menu, then **About** in the software).

Contact the International Helpdesk if the local Malvern Panalytical representative is not available:

Telephone: +44 (0) 1684 891800 or e-mail: helpdesk@malvern.com.




Note:

This help line is primarily English speaking.

Email assistance

When emailing for technical assistance, include all details that may be relevant to the problem. If possible, attach a recent data file(s) that demonstrates the problem.

You can also send a zipped archive of service-related data. To generate such support files:

1. Click the **Application**  menu and choose **Options**.
2. Scroll down to the **Service** section and click **Create Support Files** (Figure 1.1). A *PEAQ-DSC_SupportFiles* archive is automatically saved to your desktop.

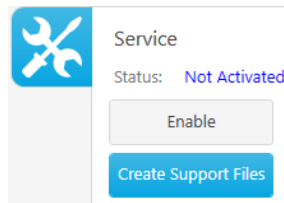


Figure 1.1 Create Support Files button

Remote support

Malvern Panalytical offers a remote support service, delivered by an Internet connection. Benefits include fast and efficient fault diagnosis, reducing downtime and costs.

CHAPTER 2 SOFTWARE OVERVIEW

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Introduction

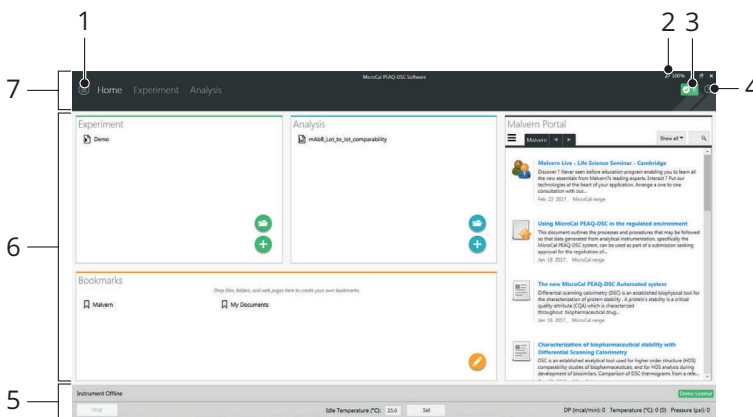
The MicroCal PEAQ-DSC is delivered with the MicroCal PEAQ-DSC software which is used to control the instrument and for data analysis. The software is preinstalled on the Controller PC.

User interface

This section provides an overview of the main components in the MicroCal PEAQ-DSC software user interface. For more details on the components in each page, see the section in which the component is introduced below.

Main screen components

Figure 2.1 shows the components in the main page of the MicroCal PEAQ-DSC software user interface.



- | | |
|---------------------------|--------------------|
| 1. Application menu | 5. Control bar |
| 2. Zoom/Font scale slider | 6. Panes |
| 3. Notifications | 7. Page navigation |
| 4. Access to Help | |

Figure 2.1 MicroCal PEAQ-DSC software main screen

Application menu

The **Application** menu provides the following:

- Access to the **Options** page where you can configure and/or modify system settings as well as view software license information
- Access to the **About** page where you can view details about the software license and software version
- **Exit** button to close the MicroCal PEAQ-DSC software



Note:

For details and instructions on how to configure system settings or to view and replace the license information, see [System options on page 16](#).

Page navigation

Use the page navigation (Figure 2.1 on the previous page) at the top of the main screen to access the **Home**, **Experiment**, and **Analysis** pages. Click the dedicated buttons from the page navigation to access the different workspaces in the **Analysis** page.

Home page

The **Home** page provides quick access to:

- The **Experiment** and **Analysis** pages.
- The **Bookmarks** section, used to drop files, folders, and web pages for quick access to frequently used items.
- The **News and Updates** section, which provides an RSS feed of news and updates from Malvern Panalytical. You can configure this section to include any RSS feed.

Experiment page

From the **Experiment** page you can:

- create and set up a sequence using the **Load** pane
- run a sequence using the **Run** pane
- view scheduled and/or pending sequences from the **Schedule** pane
- view the sequence progress from the **Plot** pane
- clean cells using the **Clean** pane

Refer to System operation on page 29 for details on using the **Experiment** page.



Figure 2.2 Experiment page

Analysis page

From the **Analysis** page you can select an experiment and progress through the analysis steps using the **Buffer**, **Baseline**, **Fit**, and **Reports** workspaces.

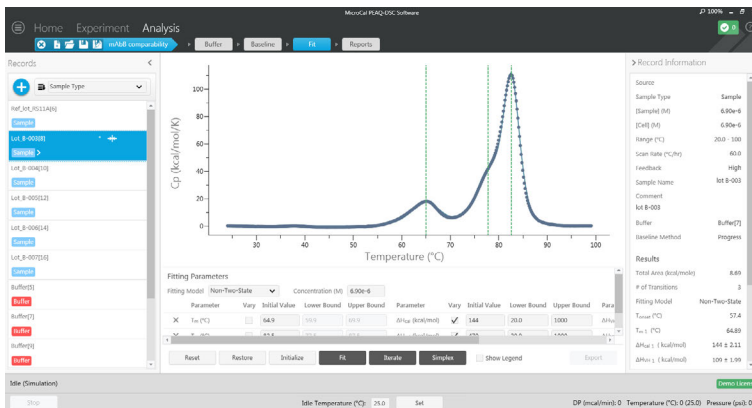


Figure 2.3 Analysis page

Refer to Analysis on page 43 for details on using the **Analysis** page.

Zoom/Font scale slider

1. Click the zoom icon to access the slider.
2. Move the slider (Figure 2.4) left to decrease the size of text and icons, or right to increase the size of text and cons.

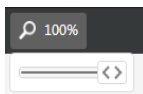



Figure 2.4 Zoom icon slider





Help

Click the **Help**  icon to open the Help system.

Notification icon

Notifications are displayed in the top-right corner of the MicroCal PEAQ-DSC software window (Figure 2.1 on page 8). Notifications are generated by the software when you need to address important information. Click the **Notification** icon (Table 2.1) to display a list of items that require your attention. The number in the icon indicates the number of alerts.

Table 2.1 Notification icons

Icon	Alert level	Color	Description
	Critical	Red	Instrument maintenance notifications have been ignored or part of the instrument is offline
	Warning	Orange	Maintenance is required or system operational messages have been ignored
	Notice	Green	No pending issues
	Information	Blue	A part will soon require maintenance

Control bar

The control bar (Figure 2.5) is located at the bottom of the MicroCal PEAQ-DSC software. Use the control bar commands (Table 2.2) to:

- View the current activity
- Stop and resume the current activity
- Monitor and set the idle temperature
- View the current DP, Temperature, and Pressure settings.

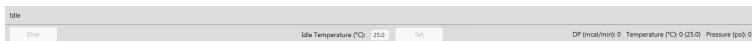


Figure 2.5 Instrument control bar

Table 2.2 Instrument control bar commands

Label	Displays
Stop	Click to stop the current instrument activity.
Idle Temperature	Enter a temperature (°C) in the Idle Temperature field. Click Set to change the idle temperature of the sample cell.
Set	Press to set the idle temperature to the specified value.

21 CFR Part 11 Overview



Note:

The 21 CFR Part 11 functionality is available only for MicroCal PEAQ-DSC users who have purchased the feature key. Contact your Malvern Panalytical representative for more details.

21 CFR Part 11 is an FDA compliance requirements specification for all computer systems that create, modify, maintain, archive, and/or retrieve electronic records required by the FDA for inspection or submission under a predicate rule. 21 CFR Part 11 compliant auditing features are available in the MicroCal PEAQ-DSC software as an option which can be activated by entering a special feature key.

Once the feature key is installed, the **Enable Audit Trail** and **Enable Electronic Signatures** features become available. With **Enable Audit Trail** selected, all user interactions with the software are recorded, and all audit features are accessible from the **View Audit Trail** button in the **Options** screen.

Refer to Enable the security features on page 18 to configure 21 CFR Part 11 features.

Access Control

The MicroCal PEAQ-DSC software can be secured at a functional level, if required, by selecting **Enable Access Control**, then configuring a permissions file in the Malvern Access Configurator (MAC). The MAC is a separate utility that is distributed with the MicroCal PEAQ-DSC software. Refer to Enable the security features on page 18.


Software updates

The MicroCal PEAQ-DSC software is preinstalled on the system. If you receive a software update notification, follow the instructions in the notification to update the MicroCal PEAQ-DSC software. Software updates are available for download on the Malvern Panalytical website.

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System options

To access information and instructions for configuring the system settings, click the **Application**  menu in the upper left corner of the screen and choose **Options**. The **Options** screen opens, as shown in Figure 3.1.

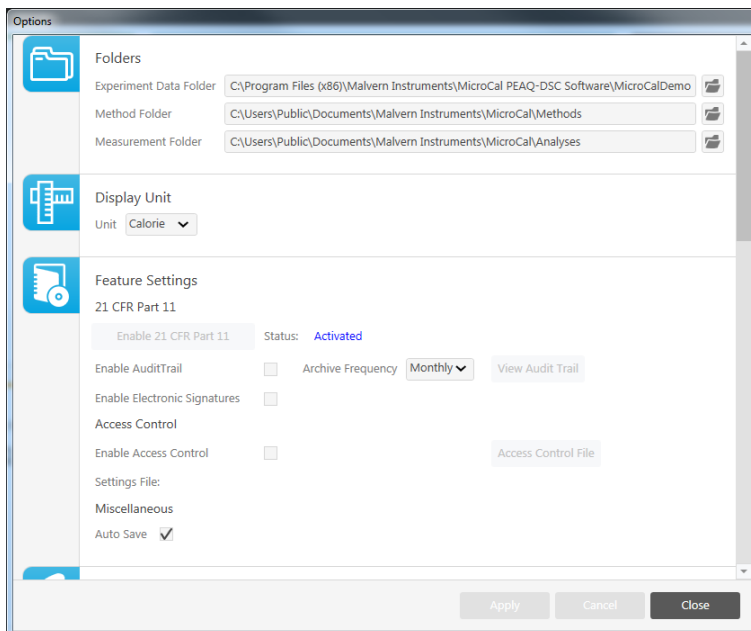


Figure 3.1 Options screen

Use the **Options** page to:

- Configure default folders
- Configure display units
- View license information and replace the license
- Enable security features
- Assign curve colors
- Configure email settings

Default folders

When you are using the system, all items are saved to a folder in a default location. Use the **Options** page to configure the default location for the following folders:

- Experiments Data Folder
- Method Folder
- Measurement Folder

The current default settings are displayed in the **Folders** section, as shown in Figure 3.2.

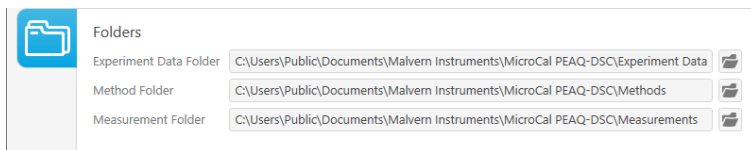




Figure 3.2 Folders section


To configure the default folder locations:

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. Click the **Browse**  icon associated with the folder you want to change and then navigate to the new folder location.
3. To create a folder, click **Make New Folder** and specify the name of the new folder.

4. Click **OK**.
5. To save the changes, click **Apply**.
6. Click **Close**.

Display unit

To configure the default display unit (Figure 3.3):

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. Click the **Unit** drop-down menu and select the unit (**Calorie** or **Joule**).
3. To save the changes, click **Apply**, then click **Close**.

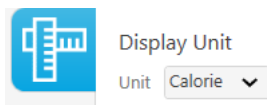


Figure 3.3 Display Unit menu

Enable the security features

The MicroCal PEAQ-DSC software uses Windows user security, by default. That is, only a user logged into the computer that is physically connected to the instrument can access the system. For many environments this level of security is sufficient.

For quality controlled environments however, the optional **Malvern Access Configurator** (MAC) adds a fully-featured security system to the MicroCal PEAQ-DSC software. The MAC provides control over an individual user's access to the features of the software. It must be enabled prior to setting up the optional 21 CFR Part 11 feature settings from the MicroCal PEAQ-DSC software.

A full description of the functionality and features of the MAC is provided in the MAC Help file.

Activate the 21 CFR Part 11

The 21 CFR Part 11 feature is optional and requires that you purchase an installation key. After installation, the following settings are available, as shown in Figure 3.4:

- Audit Trail
- Archive Frequency
- Electronic Signatures
- Access Control

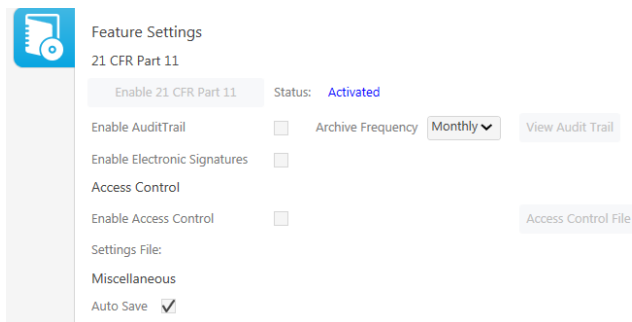



Figure 3.4 Feature Settings



Note:

The **21 CFR PART 11** feature is only available once the optional Malvern Access Configurator (MAC) has been enabled and set up in your system.

To activate the 21 CFR Part 11 feature:

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. In the **Feature Settings** section, click **Enable 21 CFR Part 11**.

3. Enter the key and click **OK**. The status changes from **Not Activated** to **Activated**.
4. To save the changes, click **Apply**.
5. Click **Close**.


Enable the audit trail

When enabled, the **Audit Trail** feature logs two types of information:

- **System audit** records all system events such as start up, shut down, record created, etc.
- **Records audit** collects the complete history of each record. It shows information related to the currently selected record(s).

As the log can grow quickly, the **Archive Frequency** feature allows you to periodically send the log items to an archive folder. The archived data is still available, but it is stored separately from the active log. The **Archive Frequency** is based on the elapsed time from the creation of the audit trail.

To enable the audit trail:

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. In the **Feature Settings** section, select **Enable Audit Trail**.
3. To specify the **Archive Frequency**, click the drop-down menu and select the desired frequency.
4. Click **Apply**, then click **Close**.

View the audit history

To view the audit trail:


1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. In the **Feature Settings** section, select **View Audit Trail** to display all non-archived system events that have been recorded (Table 3.1).
3. For edited records, click the drop-down control on the left side of the audit trail entry to view the editing information (Table 3.2).

Table 3.1 Log history information

Column	Description
Time stamp	The time in the following format: YYYY-MM-DDTHH:MM:SS (for example, 2019-09-22T12:55:35)
Action type	A self explanatory name for the action performed (for example, RecordCreated, ShutDown, Startup, RecordEdit)
User ID	The unique ID of the individual user logged onto the system who performed the action
Computer ID	The network name of the computer that accessed the system


Table 3.2 Editing records information


Item	Description
Property	The record property (for example, Sample Identifiers) that was altered
Reason for change	Records the reason for change as entered by the user
File	The file name and path of the measurement file that was modified
Modified record	The record number within the measurement file that was modified
ID	The Globally Unique Identifier (GUID) used to identify the edited record

Note:

When viewing the audit trail, you can also open the measurement file (.dmes) to view historical records of the file and to view what changes were specifically made for that measurement.

Enable the electronic signatures

The **Enable Electronic Signatures** option allows any changes performed on the system to be checked and signed. When enabled, a **Signature** icon  is shown in the **Experiments** pane of the **Analysis** page. Clicking this icon opens the **Create Signature** window where users can enter the reason for signing the record, their user name and password, and whether or not the record is locked.

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. In the **Feature Settings** section, select **Enable Electronic Signatures**.
3. Click **Apply**, then click **Close**.




Note:

For details on signing a record, see [Sign a record on page 53](#).

Enable access control

The MicroCal PEAQ-DSC software can be secured at a functional level, if required, by selecting **Enable Access Control**.

To enable access control:

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. In the **Feature Settings** section, select **Enable Access Control** to activate the security features. The **Open** dialog box opens.



Note:

The **Enable Access Control** option is only available once a security configuration file has been created using the MAC.


3. Navigate the folder in which the MAC permissions file (.xml) is located. This file is stored in the location identified with the MAC application software during its creation and export.
4. Select the file and click **Open**.
5. Click **Apply**, then click **Close**.
6. Restart the software.

Auto Save

With the **Auto Save** feature enabled, the system prompts you to enter a reason for change when you edit a measurement file from the **Analysis** page.

When using the system, all items are saved to the default folder (see Default folders on page 17).

To enable **Auto Save**:

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. In the **Feature Settings** section, select the **Auto Save** check box (Figure 3.5).
3. To save the changes, click **Apply**, then click **Close**.

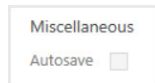


Figure 3.5 Auto Save

Curve colors

You can configure the colors for up to ten curves (labeled Curve #1 through Curve #10), the Baseline, and the Fit (Figure 3.6).

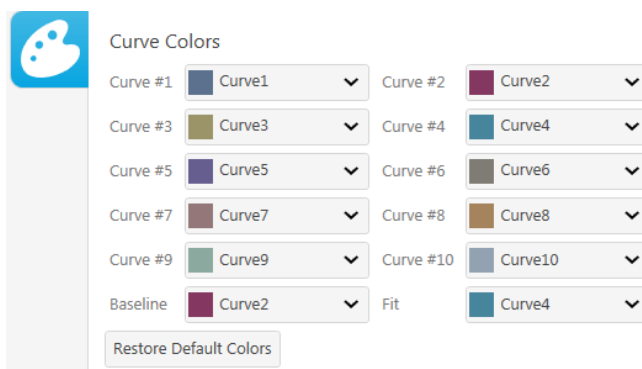



Figure 3.6 Curve Colors

To configure the curve colors:


1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. Scroll down to the **Curve Colors** section.
3. To change the color, click the drop-down menu of the item you want to change. A list of colors is shown.
4. Click the color you want to use for the selected item.
5. To use the system's default curve color settings, click **Restore Default Colors**.
6. To save the changes, click **Apply**, then click **Close**.

Software licensing


The MicroCal PEAK-DSC software does not require a license if connected to an instrument. Otherwise, you can export a license to allow other computers to use the MicroCal PEAK-DSC software without having an instrument connected.

View license information

To view the **Licensed to**, **Created by**, and **Created on** details:

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. Scroll down to view the **License** section.

To view details about the license and the license agreement:

1. Click the **Application**  menu and choose **About**. The **About MicroCal Software** information page opens.
2. Click **Details** to view the details of the active license and then click **OK**.
3. Click **License agreement** to read the license agreement and then click **OK**.

Export a license file


When the system is connected to an instrument, you can export a license. This then allows you to analyze measurement files without having an instrument connected.



Note:


The license file contains the identification of the user and the instrument from which it was generated. Do not share the license outside of your authorized user group.

To export a license within your user group:

1. Click the **Application**  menu and choose **About**.
2. To export the license, click **Export this license**.
3. Click **Yes**, then specify a file name and location where to save the license.
4. Send a copy of the **.license** file to the user without a license. On the first attempt to run the software, the system will prompt the user for the license.


Replace a license file

To replace an expired license:

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. Scroll down to the **License** section, then click **Replace License**.
3. Navigate to the folder that contains the license file (***.license**), select the file and click **Open**.
4. Click **Apply**, then click **Close**.

Email settings

To configure the **Email settings** for your system:

1. Click the **Application**  menu in the upper left corner of the screen and choose **Options**.
2. Scroll down to the **Email Settings** section and select the **Enable Email Alerts** check box to activate the Email settings (Figure 3.7).
3. Enter your Email settings. See Table 3.3 for a description of each item.



Note:

MicroCal PEAQ-DSC software only uses the Simple Mail Transfer Protocol (SMTP). When configuring the email settings, they must match the existing mail server.

4. Click **Test Email Settings** to ensure the settings are correct.
5. To save the changes, click **Apply**, then click **Close**.

Email Settings*

Enable Email Alerts?

Receiving Email

Sending Email

Email Server

Email Server Port

Sending Password

Enable SSL?

Figure 3.7 Email settings

Table 3.3 Email Alert settings

Email settings item	Description
Enable Email Alerts	When enabled, sends alerts about the system from the Email address listed in the Sending Email field to the Email address listed in the Receiving Email field
Receiving Email	Email address to which to send the Email alerts
Sending Email	Email address from which Email alerts are sent
Email Server	IP address of the host used for SMTP transactions
Email Server Port	Port number of the specified server
Sending Password	Password used to send Email alerts over the Email server
Enable SSL	Enables the use of Secure Sockets Layer (SSL) to access the specified server



Note:

If you need assistance with Email settings, contact your IT department.

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Prepare the instrument


This section describes the procedures needed to prepare the MicroCal PEAQ-DSC for an experiment.

Turn on the instrument

The On/Off power switch is located on the back of the instrument. Press the switch into the **On** (|) position to turn it on.

Start the software

Make sure to start the software and hardware in sequence for correct initialization. To start the software:

1. Make sure the instrument is on.
2. Start the computer and log in to Windows.
3. Double-click the **DSC**  icon to start the software.

Leave the power on

During frequent operations, you can leave the power on as long as the user interface program is running. The software automatically keeps the sample cell ready. Set the **Idle Temperature** (°C) to **25.0** when you leave the power on.

Periods of inactivity

When the system will not be used for extended periods of time, make sure to:

- Check that the cells are clean and empty, then fill both the sample and reference cells with deionized water.
- Close the MicroCal PEAQ-DSC software application.
- Switch off the instrument's power.

Sequence overview

Operation of the system consists of defining a sequence, loading the instrument and running the sequence.

A **Sequence** is a collection of experiments that the PEAQ-DSC instrument performs on one or more Loads. A **Load** corresponds to the material (sample, buffer or standard) inserted in the Sample and Reference cells. For each Load are programmed one or more Scans. Each **Scan** defines an experiment to be made on the Load.

When running, the sequence data is saved to a measurement file (.dmes). Once the run is completed, the data is ready for analysis.



Create a new sequence



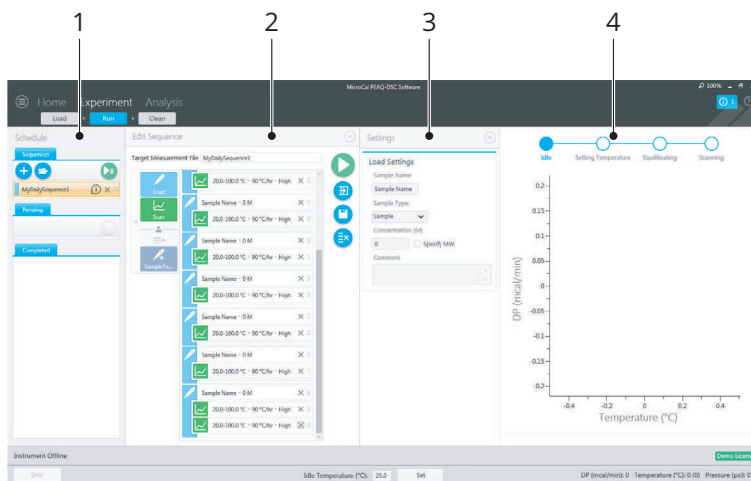
Note:

When you create a sequence, as instructed below, you define all Loads and Scans from scratch. As an alternative, you can open an existing method file and use it as a basis for your next sequence (refer to [Open a sequence on page 37](#)).

Do either of the following to create a sequence:

- From the **Home** page, click **New Sequence**  in the **Experiments** pane.
- Select the **Experiment** tab in the page navigation and click **New Sequence** .

The **Schedule**, **Edit Sequence**, **Settings**, and **Plot** panes are shown in the **Experiment** page (Figure 4.1). The **Load**  and **Scan**  icons are located in the **Edit Sequence** pane.



- | | |
|-----------------------|------------------|
| 1. Schedule pane | 3. Settings pane |
| 2. Edit Sequence pane | 4. Plot pane |

Figure 4.1 Experiment page

For details on configuring the sequence, see Set up the sequence below.

Set up the sequence

Use the **Edit Sequence** pane (Figure 4.1 above) to configure the Load and Scan settings for the sequence.



Note:

You can set up the sequence either before or after loading the instrument.



Note:




If the instrument has been idle, program several water-water and/or buffer-buffer scans at the beginning of the sequence to establish thermal history. This is critical for high-quality, repeatable, and reproducible DSC thermograms. Use the same scan rate, temperature range and feedback mode as the experimental scans.

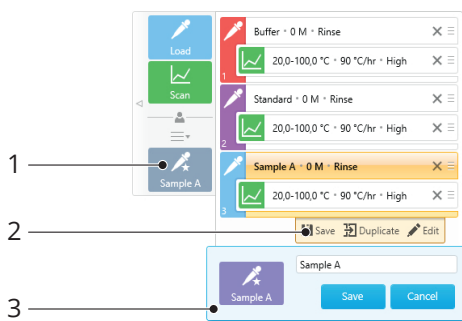
1. Enter the name of the sequence in the **Target Measurement File** field. The default name is New Sequence-*n* where *n* is a number that ensures unique names.



Note:

If you wish to append the records of an existing measurement file, enter the name of this file.

2. To adjust the Load settings, select any Load from the sequence and click **Edit** to open the **Settings** pane. Adjust the settings as needed. For details, see Load settings on page 35.
3. To adjust the Scan settings, select any Scan from the sequence and click **Edit** to open the **Settings** pane. Adjust the settings as needed. For details about the Scan settings, see Scan settings on page 36.
4. To rearrange the position of a Load or Scan, select this item from the sequence, then drag it to the desired position.
5. Do either of the following:
 - To add a new Load, click or drag the **Load**  icon into the sequence.
 - To add a new Scan, click or drag the **Scan**  icon into the Load.
 - To duplicate a Load, select this Load and click **Duplicate**. If necessary, adjust the settings.
 - To duplicate a single Scan in the Load, select this Scan and click **Duplicate**. If necessary, adjust the settings.
 - To duplicate the entire sequence, click the **Duplicate sequence**  icon. You can then edit the new sequence as you wish and run it when you want, independently from the original sequence.
 - To save a Load as a template for future use, select this Load, click **Save**. In the dialog, enter the name for the template and click **Save**. You will now see it as a gray item below the original Load and Scan items, as shown in Figure 4.2



1. Saved template
2. **Save** button from the Load
3. Save dialog

Figure 4.2 Save template

Tips:

To select multiple Loads or Scans, press and hold the **Ctrl** key on as you select each item.

To select all items in the sequence, press **(Ctrl+A)**.



To select all Loads, select the first Load and then press **Ctrl+A**.

To select all Scans, select the first Scan and then press **Ctrl+A**.

To select all Loads or Scans in-between the current Load or Scan selections, press and hold **Shift** and left-click.

To select multiple items, you can also click and drag wells in the **Tray Locations** pane.

Settings

Use the **Settings** pane to:

- Edit and configure the **Settings** for the selected Load.
- Edit and configure the **Scan Settings** and **Analysis Settings** for the selected Scan.

**Note:**

The **Analysis Settings** section in the **Settings** pane is only available when you select a second Scan of the Load.

**Note:**

For advanced information and recommendations on settings, see DSC settings on page 76.

Load settings

Click the desired Load to display the corresponding settings. Table 4.1 lists and describes the **Load Settings** section of the **Settings** pane.

Table 4.1 Load settings

Setting	Description
Sample Name	Name of the load
Sample Type	Type: Sample , Standard , or Buffer (see Table 4.2 below)
Sample Concentration	Sample concentration for the selected Load in the Sample field
Specify MW	If selected, the system specifies the molecular weight instead of the molar concentration
Load Comment	Comments about the selected load

Table 4.2 Sample type definitions

Sample type	Definition
Sample	Experiment with protein or other material in DSC cell, and matched buffer in reference cell.
Buffer	Control experiment with buffer (or water) in both DSC cells. Control experiments are used to establish thermal history and to check for DSC performance or for buffer subtraction in data analysis.
Standard	Experiment using a known sample (Reference Material, commercially available protein, or internal reference) with established DSC parameters in DSC cell, and matched buffer in reference cell. Use this sample type as a validation of PEAQ-DSC performance.

Scan settings

Click the desired Scan to display the corresponding settings. Table 4.3 lists and describes the **Scan Settings** section of the **Settings** pane.

Table 4.3 Scan settings

Setting	Description
T _{Start} (°C)	Starting temperature
T _{End} (°C)	Ending temperature
Scan Rate (°C/hr)	Scan rate
Feedback	Feedback setting: None, Low, Medium or High
Pre-Scan Thermostat (min)	Time that the instrument will hold at T _{Start} before starting a scan
Post-Scan Thermostat (min)	Time that the instrument will hold after it reaches T _{End} before continuing onto the next scan
DownScan	If selected, the scan starts at a high T _{Start} (100°C for example) and then cools to T _{End} (20°C for example). Downscans may only be scheduled on a rescan (2 nd or subsequent scan of a sample).

Analysis settings

Analysis settings are available for standard and sample Scans. Table 4.4 lists and describes the **Analysis Settings** of the **Settings** pane.


Table 4.4 Analysis settings

Setting	Description
Import	Import an analysis method settings (.dsopa) file that was developed for a previous analysis.
Reset	Reset the Analysis Settings to defaults.
Buffer Subtraction	Selected by default. When selected, the automated analysis automatically subtracts the last buffer that was successfully run in the current sequence. If a buffer has not been run previously in the selected sequence, no buffer subtraction is performed.
Baseline Type*	Type of baseline. See Baseline Type on page 85 for definitions.
Fitting Model*	Thermodynamic model applied to the data. See Fitting models on page 52 and the Help for details.
Number of Transitions*	Number of structural transitions measured in the experiment.

* Information automatically populated based on the imported .dsopa file.

Save a sequence

If you frequently run the same sequence, you can save it as a DSC method. The DSC method is saved with the .dscm file extension in the **Methods** folder.



1. After setting up the sequence in the **Edit Sequence** pane, click the **Save**  icon.
2. Rename the file in the **Save As** window. The default file name is **New Sequence**.
3. Replace the default file name by a unique name.
4. Click **Save**.

Open a sequence

You can open a saved sequence (.dscm) file if:

- you wish to use it as a basis for a new sequence, or
- you wish to append records to an existing sequence (.dmes).

Do either of the following to open an existing sequence file:

- From the **Home** page, select the experiment from the list of recent experiments or click the **Browse**  icon in the **Experiments** section and navigate to the file you want to open.
- From the **Experiment** page, click the **Load from File**  icon and navigate to the file you want to open.

For details on configuring the sequence settings, see Set up the sequence on page 32.

Load the instrument

This section provides instructions for loading the sample and buffer into the MicroCal PEAQ-DSC Sample and Reference cells. The cells are labeled **S** and **R** in the cell basin.

CHAPTER 4 SYSTEM OPERATION



Note:

Using the recommended cell loading technique is important to generate high-quality DSC data. Practice the cell filling technique with water before you load actual samples.



Note:

To view an instructional video showing how to load the instrument, click the **Play** button in the instruction video window.

You will need the following items:

- Pipette with 300 μL unused tips (pipette and tips supplied with the system)
- 250 μL of sample
- 250 μL of reference material

Perform the following steps to load the instrument:

1. Make sure that the cells are clean and empty.
2. Install a fresh 300 μL tip on the fixed volume 250 μL pipette.
3. To pick up sample using the pipette, press the plunger down until the stop, place the tip into the sample container and draw the sample material slowly.
4. Insert the tip into the Sample cell inlet port and dispense the sample slowly (approximately 50 μL per second) by slowly pressing the plunger to the stop position, as shown in Figure 4.3.
5. Repeat the steps above to load buffer into the Reference cell.



Note:

Make sure to use the matched buffer that is in the sample to achieve reproducible thermograms.




Note:

For advanced information and recommendations on samples and buffers, see *Achieve high quality data* on page 74.



Figure 4.3 Loading the instrument

Run the sequence

When you finish setting up the sequence and loading the instrument, click the **Run**  icon. The sequence is added to the pending queue and listed in the **Schedule** pane.

In the example shown in Figure 4.4, one sequence is waiting for queuing, one is running, one is queued to run, and one has completed.



Note:

Be sure the DSC cells are clean before the sequence starts.



Tip:

Hover over the  icon in the **Schedule** pane to view the remaining time and reagents for the sequence.

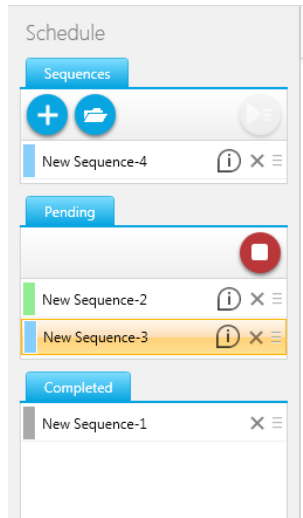


Figure 4.4 Schedule pane

The sequence progresses through stages, as shown in Figure 4.5.





Figure 4.5 Sequence stages



The Loads and Scans in the **Edit Sequence** pane show the progress of the sequence as it is running.

Manage the sequence schedule

Icons allow you to see the progress of a running sequence:

- When a Load is complete, the following check mark is shown: 
- When a Scan is complete, the following check mark is shown: 

You can manage the sequence schedule from the **Schedule** pane:

- Remove a sequence from the schedule by clicking on the **X** to the right of the sequence, if it has not yet started running.
- Stop a pending sequence from running by clicking the **Stop**  icon.
- Reorder the sequence, by clicking on a sequence, and sliding it upwards or downwards, in the desired order. Click **Yes** to confirm the new position.
- Add sequences by clicking the **New Sequence**  icon.

Clean the instrument

Clean workspace

The **Clean** workspace is located in the **Experiment** page. Follow the guided workflow to clean the instrument.

Steps include:

- Introduction
- Choose cleaning method
- Empty the cell
- Fill the cell
- Attach the manual cleaning tool
- Rinse the cell

Clean the cells

Clean the cells of the MicroCal PEAQ-DSC between scans, unless you are rescanning the same Load. Use the cleaning tools included with the instrument and 20% Contrad 70 or 14% Decon 90 as a cell cleaning detergent.



Note:

For detailed information on the cell cleaning protocols and using the Aggressive Cleaning Solution, see the Basic Guide.

CHAPTER 5 ANALYSIS

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Analysis overview

Once a sequence is completed, the data is saved to the specified DSC measurement file (.dmes). A measurement file is the destination for data coming from any number of sequences. It is appended with new data each time you run a new sequence that targets it.

The measurement file includes a separate record file (.dscx) for each Load. Records include scan data, as well as analysis. You can duplicate a record and analyze it in different ways.

The **Analysis** page shown in Figure 5.1 is where you select an experiment for analysis. The different workspaces are designed to help you progress through the analysis workflow:

- Selection of records
- Buffer subtraction
- Baseline adjustment
- Fit adjustment
- Selection of reports



Note:



For advanced information and recommendations on data analysis, see [Achieve high quality data on page 74](#).



Note:

Although you will be using the **Analysis** page to prepare your reports, reporting is covered in a dedicated chapter. Refer to [Reports on page 57](#).

Do either of the following to open a measurement file for analysis:

- From the **Home** page, click the **Browse**  icon in the **Analysis** section and navigate to the file you want to open.
- From the **Analysis** page, click the **Open**  icon in the upper left section of the page. Navigate to the file you want to open.

The default location for measurement files is:

C:\Users\Public\Documents\Malvern Instruments\MicroCal PEAQ-DSC\Measurements.

The selected measurement file is shown in the **Analysis** page, as shown in Figure 5.1 with the **Records** pane on the left, the analysis **Charts** in the center, and the **Record Information** pane on the right.






1. Records pane
2. Charts pane

3. Record information pane

Figure 5.1 Analysis page

The **Records** pane is displayed on the left side of the **Analysis** page throughout the process. Use it to:

- Add records by clicking **+** and navigating to the location where your DSC experiments are saved, then selecting the .dsc or .dscx experiment file.
- Sort records.
- View record details by hovering the mouse over the record.
- Create a new record from the same raw data as the selected record, by clicking the **Duplicate**  icon. This is useful for performing different analysis steps on the same original data.
- Edit the record name by clicking on the name. This is useful, for example, to set apart duplicated records.

- Delete the record by clicking the **Delete**  icon. You cannot delete a record that is referenced by another record. For example, a buffer that is referenced elsewhere cannot be deleted.
- Change the record from one sample type to another using the **Sample Type** drop-down menu. This will be required when importing legacy data formats.
- Average the selected records by clicking the **Average Record**  icon.

Note:

Only the buffer-subtracted, baseline-subtracted data is averaged. Certain restrictions apply due to the treatment of models with non-zero ΔC_p . You can only average records applying the same fitting model. You cannot change the fitting model of an averaged record.

The **Chart** pane in the center of each workspace shows the corresponding charts as you progress through the analysis steps.

The **Record Information** pane on the right side of the **Analysis** page shows details about the record and the results.

Import an analysis method

Use the **Import** button in the lower right section of the **Buffer** workspace to import an analysis method file (.dsopa). This imports truncation markers, baseline markers, baseline type, and fitting parameters from an exported result. For details on exporting an analysis method, see Export analysis results on page 53.

Note:

You can also preconfigure the analysis of a Scan when setting up the sequence, by importing a .dsopa file through the Analysis Settings pane (refer to Analysis settings on page 36). The resulting record then uses these analysis settings as defaults.

Restore and Reset buttons

Use the **Restore** and **Reset** buttons in the **Buffer** workspace of the **Analysis** pane as follows:

- Use the **Restore** button to restore the as-configured analysis settings.
- Use the **Reset** button to reset using the default fitting parameters. This will not affect a chosen buffer or a chosen fitting model.

For details on configuring the analysis at run time, see Settings on page 34.

Buffer subtraction

DSC experiments are run in pairs. A scan of the material of interest (Sample) is compared to a scan of the corresponding buffer. This Buffer scan is subtracted from the corresponding Sample scans before proceeding with analysis. The **Buffer** workspace lets you examine the raw data from all scans and reassign the appropriate Buffer scan if necessary.



Note:

Any of the changes described below will apply to all records selected. They will also trigger a new baseline fit, transition search, and fit to model (if specified).

Figure 5.2 shows the **Buffer** workspace in the **Analysis** page.



Figure 5.2 Buffer workspace

The charts in the **Buffer** workspace are:

- **Raw Data**, which shows the data curves for all selected records. Right-click on a Buffer curve to assign this buffer to all selected Sample records. The assigned buffer is shown as a dashed curve.
- **Buffer-Subtracted Data**, which shows the result of your buffer subtraction. You can move the vertical markers to surround the region sent for baseline calculation. This is helpful for removing the non-linear regions far from the transitions.
- **Baseline and Fitted Charts**, which provide a preview for your next analysis steps. You can collapse this pane by clicking the **Hide >** icon next to the charts title.

Assign a buffer

If **Buffer Subtraction** is configured at run time, as described in Analysis settings on page 36, the software automatically assigns the most recent, previously run Buffer scan as a buffer pairing for a given Sample scan. This is shown as a dashed curve in the Raw Data chart.

Do either of the followings to reassign a buffer scan:

- Select a buffer scan from the **Select Buffer** drop-down menu.
- In the Raw Data chart, right-click on a buffer scan and select **Assign as Buffer**.

Truncate data for baseline calculation

You can truncate data to remove baseline features such as an equilibrium tail that might affect the baseline fit. Figure 5.3 shows the **Buffer-Subtracted Data** chart.

To truncate data before baseline calculation:

1. Move the markers in the **Buffer-Subtracted Data** chart to delimit the data for baseline fit.
2. Proceed to the **Baseline** workspace.

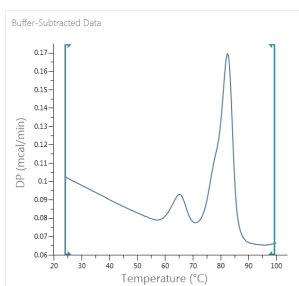


Figure 5.3 Buffer-subtracted data chart

Baseline adjustment

If necessary, adjust the baseline using the **Baseline** workspace, as shown in Figure 5.4.

To trigger a new transition search and a new fit to model (if specified), adjust the **Baseline parameters** and/or redraw the baseline, as per described in the following sections.



Note:

Any changes described in this section will apply to all records selected.

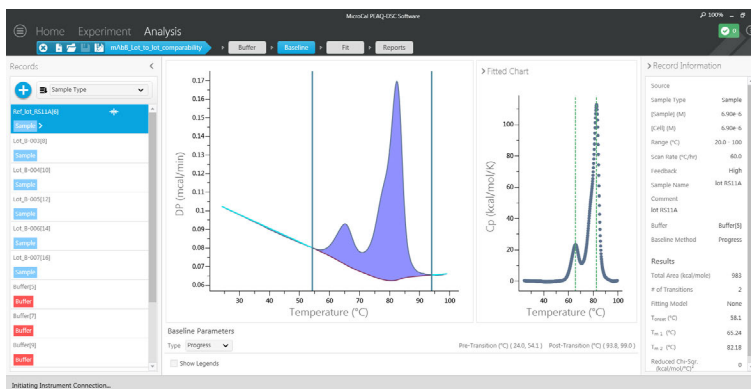


Figure 5.4 Baseline workspace

Redraw the baseline

Use the **Baseline Parameters** section in the **Baseline** workspace of the **Analysis** pane to:

- View or modify the **Pre-Transition** temperature
- View or modify the **Post-Transition** temperature
- View the datapoints
- Specify the baseline type
- Show/hide legends in the displayed charts

The two markers in the baseline chart define the innermost limits of the transition baseline region. The data that are outside of the markers help define the baseline.

To redraw the baseline:

1. From the **Analysis** page, select the **Baseline** workspace.
2. In the **Adjust Baseline** chart, select a marker you want to move.
3. Move the marker as needed to redraw the baseline.



Note:

As you move the marker, the corresponding datapoint is shown below the chart. Release the mouse button to set the value to the nearest datapoint.

4. If necessary, change the **Type** in the **Baseline Parameters** section.

Fit adjustment

If the generated analysis result is not satisfactory, you can manually adjust the fitting parameters (Figure 5.5). This can be the case when too many fitting parameters are varied, when using more complex models, or if erroneous concentrations have been entered.



Note:

Any changes described in this section will apply to all records selected.

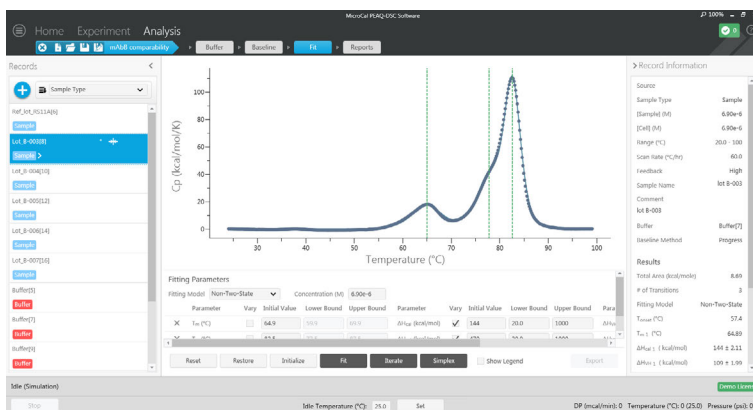


Figure 5.5 Fit workspace

Add a transition guess

Adding a transition guess overrides the results of the automatic transition detection. The software then uses that transition guess as a seed to its fitting algorithm.

To add a transition guess:

1. From the **Analysis** page, select the **Fit** workspace.
2. In the fitted data chart, right-click on the curve to add a transition guess. The parameter values are displayed for each transition guess that you add.
3. To remove a guess, click the **X** that corresponds to the transition guess in the **Transition Estimates** section.
4. Click **Fit**.

Adjust the transition estimates

By default, a thermodynamic model is not applied to the data. Instead, transitions are detected and reported as Transition Estimates. If you want to apply a model to the data, select a fitting model to configure a fitting session.

The MicroCal PEAQ-DSC software provides three primary models to which you may fit your data. All three models use the Levenberg-Marquardt non-linear least-square method, but differ in the number of parameters involved, as shown in Table 5.1. The parameter definitions are:

- T_m is the thermal midpoint of a transition
- ΔH_{cal} is the calorimetric heat change
- ΔH_{VH} is the van't Hoff heat change
- B_0 and B_1 define the slope and intercept of the low-temperature baseline segment

Table 5.1 Fitting models

Model	Parameters
Model 1: Two-State	$T_m, \Delta H_{cal}$
Model 2: Non-Two-State	$T_m, \Delta H_{cal}, \Delta H_{VH}$
Model 3: Two-State, $\Delta C_p \neq 0$	$T_m, \Delta H_{cal}, C_p, B_0, B_1$

**Note:**

Refer to the [Help](#) for details on fitting models and calculations.

Export analysis results



You can export the results of the analysis to an analysis method file (.dsopa). This can either be applied to another record or used to preconfigure analysis on a Scan at run time (refer to Analysis settings on page 36).


To export results to an analysis method file:

1. From the **Analysis** page, select the **Fit** workspace.
2. Select the file you want to export.
3. Click the **Export** button in the lower right section of the workspace.
4. In the **Save As** window, navigate to where you want to save the file and enter the file name in the **File name** field.
5. Click **Save**.


Sign a record

There are two types of signatures:

- Non-locking signatures can be appended multiple times. The **Signature**  icon displays as blue if there is at least one non-locking signature. If the record is edited after a non-locking signature is applied, all signatures are erased.
- Locking signatures allow a one-time only signature and then the record is locked. The **Signature** icon is displayed as a gold padlock . No further edits are allowed to a locked record.

To view details about the record's signatures, as shown in Figure 5.6, hover the mouse over the **Signature**  icon.

**Note:**

The electronic signature feature must be enabled to display the **Signature**  icon in the **Records** pane of the **Analysis** page. For more information, see [Enable the security features on page 18](#).

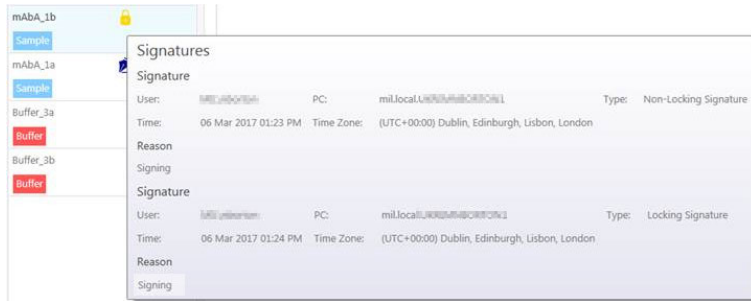



Figure 5.6 Signatures detail

To sign a record:

1. From the **Analysis** page, select the **Fit** workspace.
2. Click the **Signature**  icon in the **Records** pane.
3. In the **Create Signature** window (Figure 5.7), enter the reason for the signature, your user name and your password.
4. To lock the record (no further editing is permitted), select the **Locking Record** check box.
5. Click **Create**.

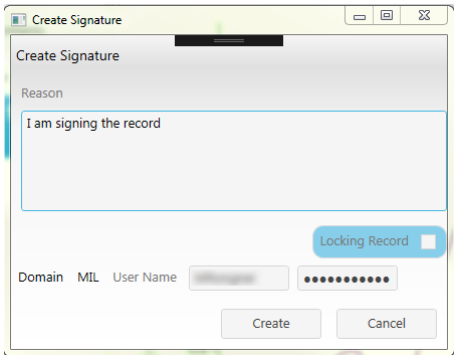


Figure 5.7 Create Signature window

CHAPTER 6 REPORTS

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Introduction

Reports help to aggregate all of the data generated in a measurement to enable you to graphically analyze measurement data.

The MicroCal PEAQ-DSC software reports consist of data sections that are shown together for easy on-screen analysis or printing. Some default reports are supplied with the software to demonstrate basic reporting functionality. Use them to design your own customized reports.

You can view the reports from the **Reports** workspace in the **Analysis** page, as shown in Figure 6.1.

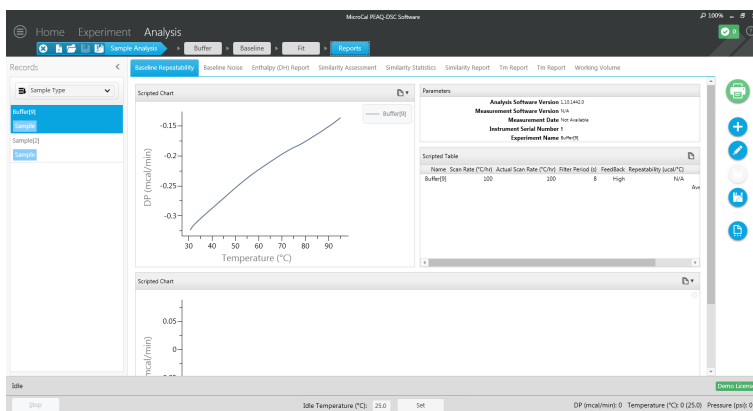


Figure 6.1 Reports workspace

Most users only need to select, print or export the content of a report as shown in Use reports below. To create and edit reports, refer to Build reports on page 61.

Use reports

Most users will only work with existing reports, to select and print or export them as described in this section. To create or modify reports, see Build reports on page 61.

Select a report

Reports are shown in the **Reports** workspace of the **Analysis** page. Each reports has an individual tab at the top of the workspace (Figure 6.2). The tab of the currently shown report is highlighted in blue. Click the corresponding tab to show a different report.

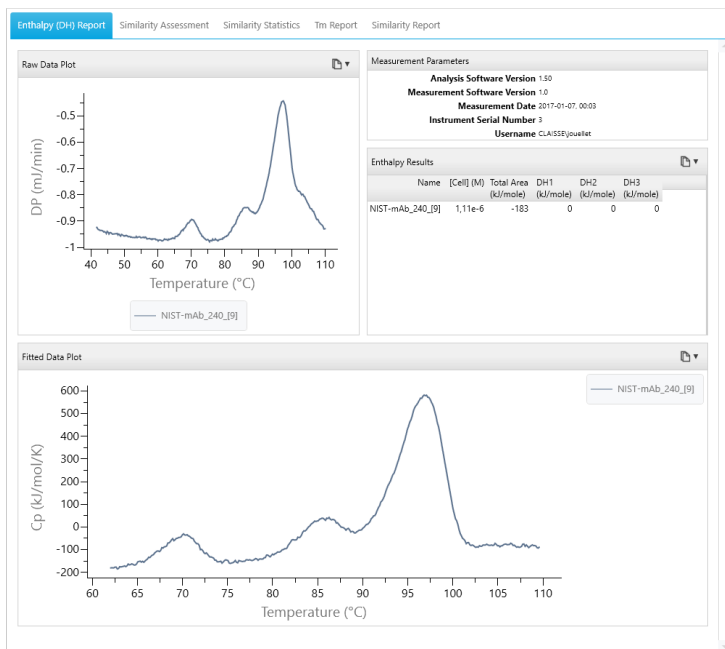






Figure 6.2 Current report in workspace

To add, delete or reorder reports in the workspace:

1. Click the **Select Reports**  icon from the right-hand side menu.
2. In the **Select Reports** window (Figure 6.3), use the  and  buttons to move reports between the **Available** and **Selected** sections.
3. Once the **Selected** section lists the desired reports, use the  buttons to arrange the order of the reports as needed.
4. Click **OK**.

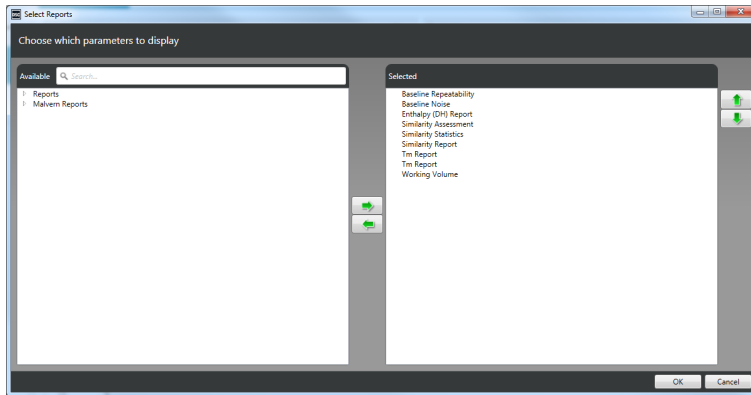


Figure 6.3 Select Reports window

Print a report

To print a report:




1. From the **Records** pane, select the records you want to include in the report. Do either of the following:
 - To select multiple records, press and hold the **Ctrl** key, while selecting each record.
 - To select a range of records, press and hold the **Shift** key, while selecting the first and last records in the range.
 - To select all records, press **Ctrl+A**.
2. Select the report to print, then click the **Print**  icon. A preview of the report is shown.
3. Click the **Print**  icon above the preview controls (Figure 6.4) and select the printer on which to print the report.



Figure 6.4 Print report

Export report data

Some of the panes in the **Reports** workspace include the **Export menu**  (Figure 6.5) where you can choose to export the content of the pane. Depending on the type of information, you can save content as data, as an image or as a table.

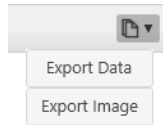



Figure 6.5 Export menu

Build reports

Create a report

You can create and organize your own customized reports from the **Reports** workspace.

To create a report:

1. From the **Analysis** page, select the **Reports** workspace. Open the desired measurement if not already shown. For details, see Analysis overview on page 44.
2. From the right-hand side menu, click  to display a blank report.
3. Refer to Edit a report on page 64 to add content to your new report.

Report components

Use this section as reference when editing a new or an existing report. For details, see Edit a report on page 64.

Header

The **Header** section of the report includes the report name, company name, and logo (see Figure 6.6). By default, the report is titled **New report** whereas the company name and logo are those of the Malvern brand.

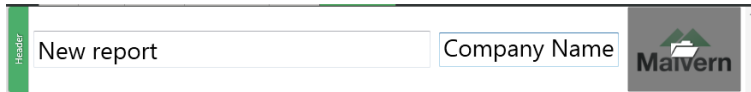


Figure 6.6 Report Header section

To change a header component, click the field you wish to edit.



Note:

The company name and logo are global settings and are used for all reports. You do not need to edit these each time you create a report.

Footer

The **Footer** section of the report contains Malvern Panalytical information, the analysis name, the report creation and printed date, as well as the software version. You cannot edit the text in the footer section.

Containers and widgets

Report data are shown in panes called *containers*. Containers are frames that define the layout of a report and host data components, or *widgets*. When editing a report, you select the container format and layout, then assign a widget to each container. This fills the report with specific types of data.

Figure 6.7 shows the widgets you can select to populate the report. Table 6.1 lists and describes the widgets.

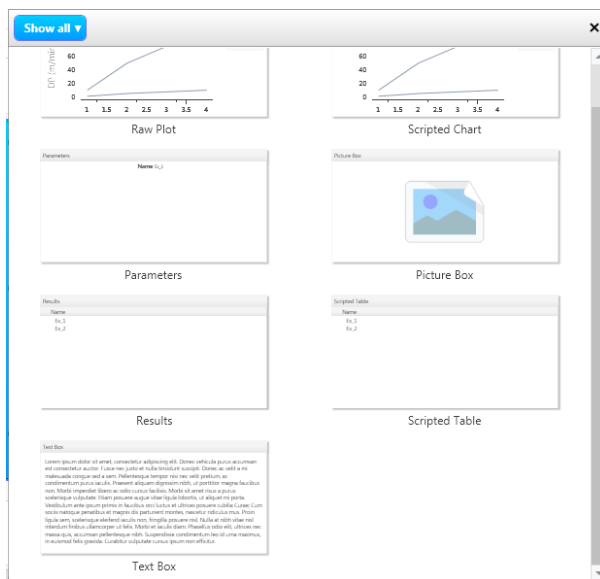


Figure 6.7 Widgets

Table 6.1 Widget descriptions

Widget name	Description	Options
Parameters	Grid populated with parameters	See page 65
Results	Results of the selected experiments	See page 66
Text Box	Box for adding notes and annotations to reports	See page 67
Scripted Chart	Chart with selectable contents	See page 67
Picture Box	Box that can be used for images and logos	See page 68
Scripted Table	Table with selectable contents	See page 69
Signature	Table that shows the electronic signatures (if applicable) that are associated with the selected records	See page 70







Edit a report



Note:

For details on working with widgets, see [Widget properties on the facing page](#).





To edit a report:

1. At the top of the **Reports** workspace, select the tab of the desired report.
2. Click the **Edit**  icon from the right-hand side menu.
3. To change the header, click the corresponding field.
4. Do either of the following to change the layout of the report:
 - To add a container, click the desired layout from the container addition menu , where the new panes should be inserted.
 - To choose a different layout for a container, click the  icon on the right of this container.
 - To delete a container and its widgets, click the  icon on the right of this container.
 - To vertically resize a container, click  and move the frame border up or down as needed.
5. Do either of the following to select a widget:
 - Click the blue background of an empty container, then click the desired widget from the pop-up window.
 - Click the  icon on the left of an existing widget, then click the desired widget from the pop-up window.



Note:



At first, widgets are blank, you must edit the widget properties to show data.

6. Click the **Options menu**  icon to edit the widget. Do either of the following:
 - Edit the **Title** field as needed or use the default title.
 - Set the widget's content as needed. Refer to Widget properties below.
 - Use the **Set** button to temporarily apply the settings.
 - Click **Restore** to restore the widget properties to its original settings.
7. Repeat the above steps until the contents of the report fit your requirements.
8. Click the **Save**  or **Save As**  icon to save changes.
9. Click the **Edit**  icon from the right-hand side menu to exit the report designer.

Widget properties

Parameters widget

Use the **Parameters** widget to select and show a grid populated with parameters. When you first add the **Parameters** widget, there are no parameters selected.

1. Click  to show the options menu.
2. Use the slider to vary the width ratio and to move the label and value left and right as needed.
3. Select the **Show name and unit together** check box to put the unit with the label or the value.
4. Click , then **Select Parameters** to open the **Select Parameters** window (Figure 6.8) where you can choose from available parameters to include in the report.

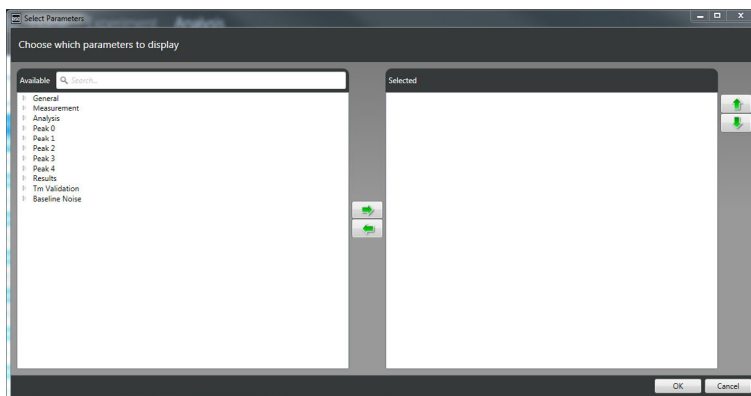






Figure 6.8 Select Parameters window

To add, delete or reorder parameters:

1. Use the  and  buttons to move items between the **Available** and **Selected** sections.
2. Once the **Selected** section lists the desired items, use the  buttons to arrange the order of the items as needed.
3. Click **OK**. The selected items are shown in the widget.

Results widget

Use the **Results** widget to include results of the selected experiments in the report.

1. Click , then **Select Parameters** to open the **Select Table Columns** window (Figure 6.9).
2. Choose which parameters to show in the **Results** widget.

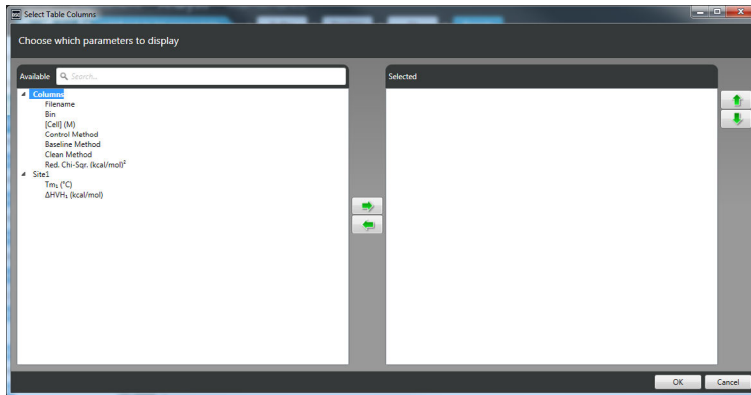





Figure 6.9 Select Table Columns window

To add, delete or reorder parameters:


1. Use the  and  buttons to move items between the **Available** and **Selected** sections.
2. Once the **Selected** section lists the desired items, use the  buttons to arrange the order of the items as needed.
3. Click **OK**. The selected items are shown in the widget.

Text box widget

Use the **Text box** widget to enter basic text and comments about the report, measurement, or other relevant information.

Scripted Chart widget

Use the **Scripted Chart** widget to include a chart with selectable contents. When you first add the **Scripted Chart** widget, there are no scripts selected.

1. Click  to show the options menu.
2. Click **Chart Options** to open the **Edit Chart Settings** window.
3. Click the **View** drop-down menu to select an installed script.

CHAPTER 6 REPORTS

4. Click **Edit Script** to modify, load, or create the calculation to include in the report. When you click **Edit Script**, the **New Calculation** window is shown (Figure 6.10). Use the controls in the **New Calculation** window to create, open or save a calculation, or to check the calculation syntax.



Note:

Click the **Help ?** icon from the right-hand side menu of the **Reports** workspace to access the PEAQ-DSC Scripting help.

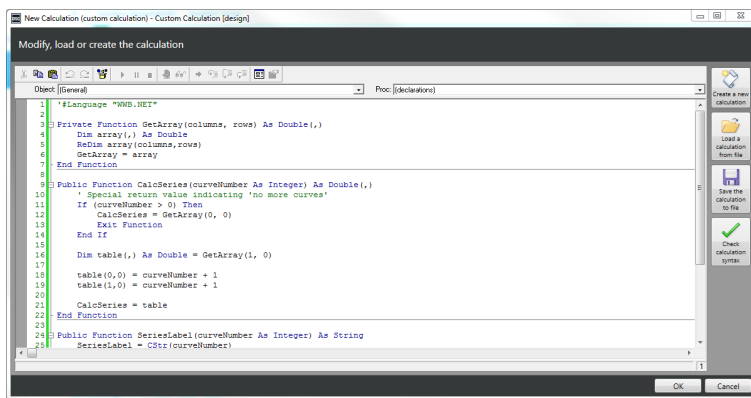




Figure 6.10 New Calculation window


Picture box widget

Use the **Picture box** widget to insert an image in the report.

- To insert an image, click , then **Select picture** to open Windows Explorer and select an image.
- To align the image position, click  to select the alignment and the scaling factor, then click **Set**.
- To restore the settings and clear the Picture Box, click **Restore**.

Scripted Table widget

Use the **Scripted Table** widget to include a table with selectable contents in the report. When you first add the **Scripted Table** widget, there are no scripts.

1. Click  to show the options menu.
2. Click the **View** drop-down menu to select an installed script.
3. Click **Select Properties** to open the **Select Table Columns** window (Figure 6.11) and choose which parameters to show in the **Results** widget.

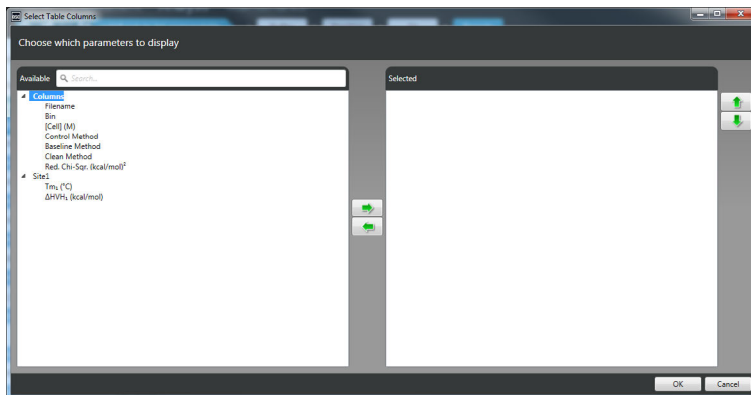


Figure 6.11 Select Table Columns window

4. Click the **Edit Script** option to modify, load, or create the calculation to include in the report. For details, see Scripted Chart widget on page 67.




Note:

Click the **Help**  icon from the right-hand side menu of the **Reports** workspace to access the PEAQ-DSC Scripting help.

Signatures widget

Use the **Signatures** widget to include electronic signatures that are associated with the selected records in the report. This widget only contains data if the electronic signatures feature is enabled.

1. Click  to show the options menu.
2. Uncheck the **Show All Signatures** box if you only wish to include the latest signatures for each selected record. This box is checked by default, meaning that the widget will include all signatures.
3. Check the **Short timezones** box if you only wish to include the timezone difference from GMT. Uncheck the box to also include the timezone location.

Save a report

Do either of the following to save a report:

- To save the report, click the **Save**  icon.
- To save the report with a different name, click the **Save As**  icon.

After you have saved a new report or saved a report under a different name, a new tab shows this report in the **Reports** workspace.

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Differential Scanning Calorimetry (DSC)

DSC is a technique for understanding the stability of proteins and other biomolecules. It has wide application in protein engineering, rational drug design and biopharmaceutical production, where developing stable proteins is a critical goal. DSC is a key thermal analysis technique that measures the heat changes that occur in the biomolecule during a controlled increase or decrease in temperature, making it possible to study materials in their native state.

DSC enables:

- Characterization and selection of the most stable proteins/potential candidates in biotherapeutic development
- Ligand interaction studies
- Quick optimization of purification and manufacturing conditions
- Easy, quick determination of optimum conditions for liquid formulations
- Quick stability-indicating assay of the target proteins for use in screening

DSC measures the enthalpy (ΔH) and transition midpoint (T_m) of thermally-induced structural transitions in solution. This information gives valuable insights into factors that stabilize or destabilize proteins, nucleic acids, micellar complexes and other macromolecular systems. The data are used to predict shelf-lives, develop purification strategies, characterize and evaluate protein constructs or other biotherapeutic entities, and to rank the affinities of ligands to a protein target in small molecule drug discovery programs.

Note:



A biomolecule in solution is in equilibrium between the native (folded) conformation and its denatured (unfolded) state. The measured T_m provides a quick and easy indication of stability. The higher the T_m , the more stable the biomolecule.

How DSC works

The well-defined structures formed by proteins and other macromolecules undergo temperature induced conformational changes, such as unfolding. These changes result in the absorption of heat as a result of the redistribution of non-covalent bonds. Differential scanning calorimeters measure this heat uptake, as shown in Figure 7.1.

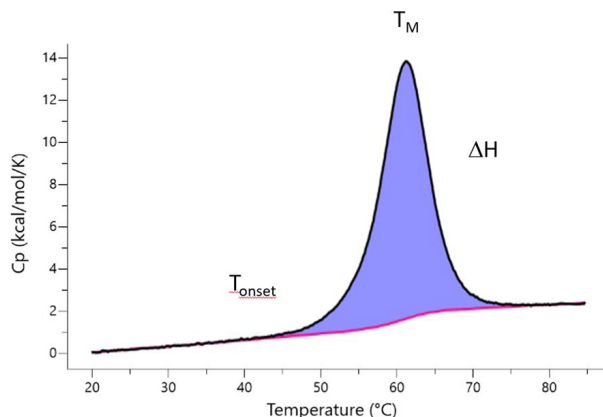


Figure 7.1 Typical DSC thermogram

When the DSC experiment starts, the protein is primarily folded in its native conformation. With increasing temperature the protein eventually begins to unfold (T_{onset}) and the heat capacity (C_p) increases. When 50% of the protein is folded and 50% is unfolded, the C_p reaches its maximum value. This is the T_m . Above the T_m , the protein is primarily denatured and, at the end of the DSC experiment, all of the protein is unfolded.

The thermal core of the MicroCal PEAQ-DSC cell unit contains both a reference and sample cell, within an insulating jacket. The reference cell is filled with buffer and the sample cell is filled with sample. The system maintains these two cells at the same temperature. During a measurement, the cells are heated at a constant scan rate. As the sample molecule unfolds, it absorbs heat, creating a temperature difference between the sample and the reference cell. The system translates the resulting heat flow between the cells as Differential Power (DP).

Feedback modes allow for a faster return to equilibrium using heaters to actively balance the two cells at the expense of increased signal noise. Alternatively, a passive mode may be used to let the cells equilibrate through the existing conduction paths.

Data generation and analysis

The enthalpy of protein unfolding is the area under the DSC peak and has units of kcal/mol or kJ/mol. Thermodynamic models can be fitted to the data to obtain the Gibb's free energy (ΔG), the calorimetric enthalpy (ΔH_{cal}), the van't Hoff enthalpy (ΔH_{vH}) and the change in the heat capacity (ΔC_p) associated with the transition. Refer to Analysis on page 43.

Achieve high quality data

This section provides recommendations and advanced information to help you design better protocols and experiments as well as refine your analysis.

Sample preparation

For best results, use a purified protein or biopolymer of interest as contaminating material can affect the thermogram. If your final purification step is gel filtration or size exclusion chromatography with the same buffer that is used for DSC, the sample needs no further preparation. In all other cases, use dialysis, a desalting column, or another buffer exchange method to prepare the sample in the appropriate buffer for the DSC study (see Buffers for DSC below).

DSC results are affected by buffer, pH, excipients, etc. Save the buffer from sample dialysis or buffer exchange to use it for the reference cell, buffer-buffer scans, and sample dilution. It is important that the DSC reference cell contain the matched buffer that is in the sample, to achieve reproducible thermograms.

Remove particulate matter from the sample before filling the DSC cell.

Buffers for DSC

MicroCal PEAQ-DSC cells and other system components are compatible with biological, aqueous buffers between pH 2 and pH 12. See the Basic Guide for a list of tested compatible chemicals. Contact Malvern Panalytical if you have questions about using a specific chemical or additive.

If you need to include a reducing agent, Malvern Analytical recommends 2-mercaptoethanol (2-ME) or Tris(2-carboxyethyl)phosphine (TCEP), instead of dithiothreitol (DTT).

For non-aqueous solvents, check the list of tested compatible chemicals. If the solvent of interest is not listed, use published chemical compatibility charts to check the resistance of the wetted materials to your solvent.

Avoid using buffer or samples with high viscosity (due to glycerol, other additives, or high sample concentration), as bubbles would be introduced into the DSC cells while filling. Bubbles can cause a mismatch in the heat capacity measurements and result in non-reproducible data.

Sample concentrations

Prior to the DSC experiments, accurately measure the sample concentration and determine molar concentration, based on molecular weight. Molar concentration is used to calculate enthalpy from the DSC thermogram. If any protein in the original solution is already denatured, the heat change will be less than expected.

The recommended range for proteins and other biopolymers is 0.2 mg/mL to 2 mg/mL. Depending on the sample, you can obtain good thermograms with lower or higher concentrations. During method development, make sure to optimize sample concentration for DSC. For example, T_m screening studies may need less protein than other characterization studies. Larger molecular weight proteins tend to need lower concentration compared to smaller proteins, since larger proteins have more non-covalent bonds to break during thermal denaturation.

Using a lower sample concentration tends to result in noisier thermograms, a poor signal to noise ratio, or heat changes that are insufficient for detection by the DSC. This can cause non-reproducible data as well as a greater variance and standard deviation for enthalpy and other DSC parameters.

Higher concentrations tend to result in a lower apparent T_m if the protein aggregates with thermal denaturation, or a higher apparent T_m if the protein forms a stable oligomer at higher concentrations.



WARNING!

Do not use very high protein concentrations. Above 10–20 mg/mL, proteins can aggregate or gel, which causes excessive contamination of the cells. Excessive contamination will require aggressive cell cleaning (see the Basic Guide) or a visit by a service engineer.

Storage temperature

Be sure to store the samples at an appropriate temperature prior to DSC analysis.

DSC settings

T_m and DSC thermograms can be dependent on sample concentration, scan rate and feedback mode. You must decide if resolution, sensitivity or throughput is most important for your DSC experiments, and balance the experimental conditions accordingly. Following are some recommendations on critical DSC settings for data quality. See Settings on page 34 for general information on MicroCal PEAQ-DSC settings.

For comparisons of the DSC thermograms as part of a characterization study (such as T_m screening or similarity studies) be sure all experimental settings are the same, including scan rate, temperature range, feedback mode, and pre-scan thermostat.

DSC temperature range

The total temperature range of the instrument is 1–130°C. Start the scan 10–20°C below the first T_m and end the scan 10–20°C above the final T_m . If you heat proteins much higher than the T_m , the protein is likely to precipitate in the DSC cell, making cell cleaning more difficult.

DSC scan rates

The maximum scan rate is 240°C/hr. Typical scan rates are:

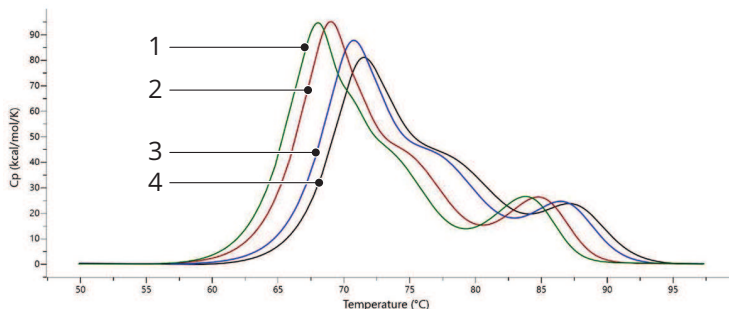
- 60–90°C/hr for protein studies
- 10°C/hr for lipids and other materials that have DSC transitions of less 5°C

To optimize DSC scan rate, test for reversibility of the thermal transition—typically by reheating the sample and observing an identical transition—and run a series of scan rates. The optimal scan rate does not result in a distortion of the transition characteristics and is reversible.

When studying irreversible systems, make sure to use the same scan rates for each measurement that you wish to compare. The DSC thermogram will highly depend on scan rate (see

Figure 7.2) because the denaturation process is rate limited. For these comparative studies, Malvern Panalytical recommends the fastest scan rate to minimize experimental time and to increase signal to noise ratio (typically, the faster the scan rate, the larger the signal).

For antibodies and other proteins with multiple transitions, choose the scan rate that maximizes the differences in the thermograms that you will be comparing.



1. 60°C/hour
2. 90°C/hour

3. 180°C/hour
4. 240°C/hour

Figure 7.2 Antibody X thermograms at 4 scan rates

Feedback modes

Feedback mode also impacts resolution and sensitivity. Faster (high) feedback modes can increase resolution of multiple transitions but decrease signal to noise ratio. Suggested feedback modes are:

- Proteins: none or low
- DNA and RNA: low or medium
- Lipids: high

Pre-scan thermostat

Longer pre-scan thermostat times tend to result in more reproducible thermograms.

Best practices

Always consider the following when operating your system.

Keep DSC cells clean

Do regular maintenance as instructed in the Basic Guide to keep the cells clean.



Note:

Cleaning is considered part of maintenance. Failure to keep the cells clean and properly maintained can void the instrument warranty.

To help preserve clean cells in your routine use of the system,

- Before an experiment, free all solutions of visible particles by centrifuging or filtering as particles or precipitates can be particularly difficult to remove from the DSC cells.
- Whenever possible, reduce or eliminate precipitation in the DSC cells by using lower sample concentrations, and heating the samples at a maximum of 5-10°C higher than the last T_m .



Note:

Some proteins require more rigorous cleaning protocols, regardless of protein concentration or heating temperature.

- If analyzing samples that tend to precipitate after thermal denaturation, run a cleaning step to remove the precipitated material from the cell before the next sample is loaded.
- Do not let samples sit in the cells for a long period of time.
- Always rinse the cells with water after using the instrument.
- If the system will be used within the next two weeks, make sure the DSC cells are clean and fill them with deionized water to prevent bacterial growth.
- If the system will not be used for an extended period of time (more than two weeks), make sure the DSC cells are clean and dry to prevent bacterial growth.

CHAPTER 7 THEORY OF OPERATION

Regularly assess the cleanliness of the DSC cells. Evidence of dirty cells includes:

- In raw thermograms, matched water-water or buffer-buffer scans are not repeatable (see Figure 7.4 compared with Figure 7.3).
- In raw thermograms, matched water-water or buffer-buffer scans are below 0 mcal/min (see Figure 7.4 compared with Figure 7.3).
- DP values drift negatively from first to last sample in sequence (see Figure 7.4 compared with Figure 7.3).
- Water-water or buffer-buffer scans do not become reproducible when establishing thermal history.
- Thermograms of replicate samples or replicate validation samples are not reproducible and show changes in expected T_m , enthalpies, etc.
- DSC cells are filled with bubbles.

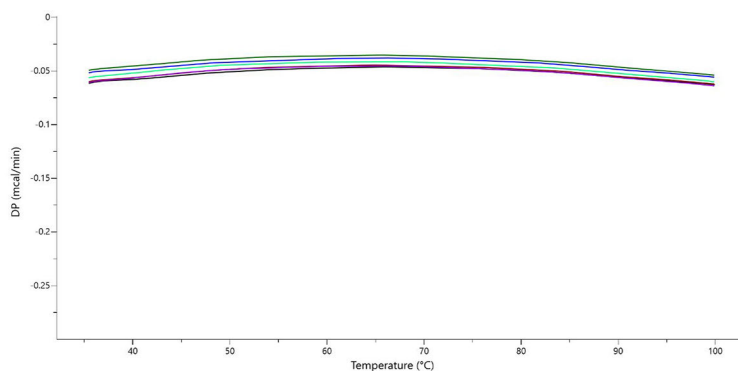


Figure 7.3 6 buffer-buffer scans with clean cells

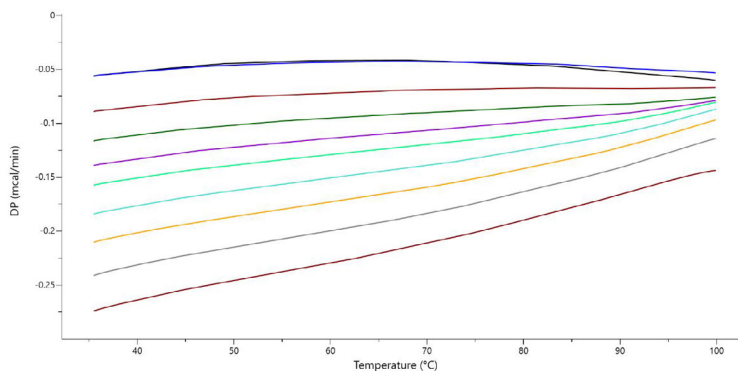
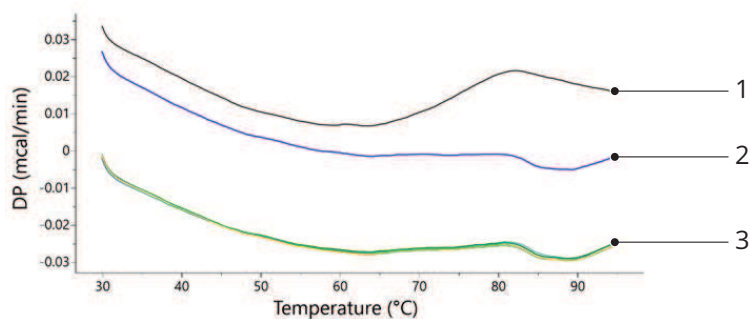


Figure 7.4 10 buffer-buffer scans with dirty cells (first scan on top line)

Establish thermal history

If the instrument has been idle, make sure to establish a thermal history before you expect reproducible sample scans. Otherwise, the first thermogram of your sequence will have a different shape or DP position than the following thermograms, reducing the repeatability of your DSC data.

To establish thermal history if the instrument has been idle, program two to five scans (Figure 7.5). Set a water-water or buffer-buffer scan as the first experiment in the sequence, with the same experimental parameters as the following scan(s) in the sequence.



- | | |
|--|--------------------|
| 1. Scan 1 (first scan after idle time) | 3. Scans 3,4 and 5 |
| 2. Scan 2 | |

Figure 7.5 Water-water scans to establish thermal history

Preserve thermal history

When doing a series of DSC experiments in a sequence, clean the cells and fill the next sample in-cycle, i.e. while the cells are cooling. This will preserve the thermal history of the instrument.



Note:

The cooling phase typically lasts 15 to 20 minutes, depending on the temperature range.

Program buffer-buffer scans during a sequence

Malvern Panalytical recommends that you perform frequent buffer-buffer scans, with the same buffer as the samples, and same experimental parameters as the sample scans. The matched buffer-buffer scans are used to:

- correct the thermograms for instrument background during data analysis
- analyze the performance of the DSC
- analyze baseline stability
- analyze the effectiveness of the cell cleaning procedures

Validate standards and DSC performance

Malvern Panalytical recommends that you regularly perform DSC scans of validation standards or known samples with well-characterized DSC thermograms, T_m and enthalpies. These scans help assess the DSC performance, and check that the DSC cells are clean. Run your validation standards at frequent intervals during a sequence, for example after every 10th sample scan. Possible validation samples include:

- MicroCal PEAQ-DSC Reference Material
- Commercially available proteins such as RNase, lysozyme, carbonic anhydrase, and NISTmAb reference protein
- Internal standards such as a reference lot of material

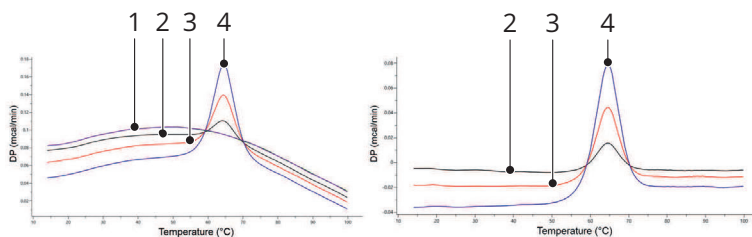
Evaluation of DSC data quality

To obtain high-quality DSC thermograms you need to:

- prepare the samples correctly in the buffer of interest
- use the proper technique when filling the sample and reference cells (see Load the instrument on page 37)
- insert the matched buffer in the reference cell for every sample
- run a buffer-buffer scan using the matched buffer
- make sure the DSC cells are clean
- establish thermal history (see Best practices on page 80)
- clean and fill the DSC cells in-cycle

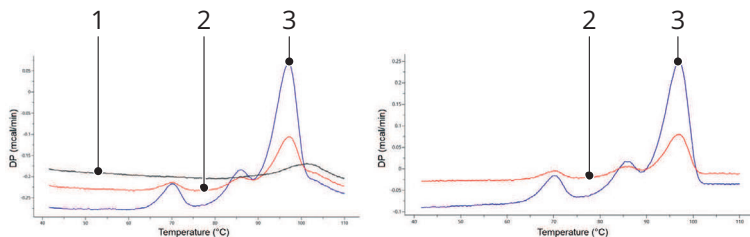
Figure 7.6 and Figure 7.7 show high quality DSC thermograms with raw data on the left and buffer-subtracted data on the right.

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- | | |
|------------------------|--------------|
| 1. Buffer-buffer scans | 3. 0.5 mg/mL |
| 2. 0.2 mg/mL | 4. 1 mg/mL |

Figure 7.6 DSC scans of Protein X before (left) and after (right) buffer-buffer baseline subtraction



- | | |
|------------------------|--------------|
| 1. Buffer-buffer scans | 3. 0.5 mg/mL |
| 2. 0.17 mg/mL | |

Figure 7.7 DSC scans of NISTmAb reference before (left) and after (right) buffer-buffer baseline subtraction

In high quality raw thermograms (on the left in Figure 7.6 and Figure 7.7):

- Buffer-buffer scans are reproducible and parallel, close to 0 mcal/min in DP (Y axis value).
- Sample scans are parallel to buffer-buffer scans.
- Sample scans are in the same temperature range as the buffer-buffer scans before and after the transition(s).
- Sample scans have a lower DP (more negative Y axis value) compared to the buffer-buffer scan.

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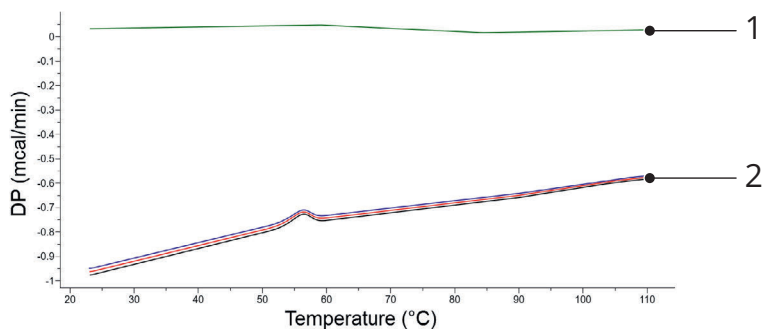
- A DP difference in sample versus buffer-buffer is greater (more negative value) for higher protein concentrations.
- With good buffer matching, this DP difference should be approximately 0.02 to 0.2 mcal/min below the buffer-buffer scan for every 1 mg/mL of protein.
- Transition peak(s) are evident and detectable above any noise in the thermogram.
- Transition areas are larger for higher sample concentrations.
- Replicate scans of the same sample are reproducible and parallel to each other.

In high quality thermograms following buffer-buffer baseline subtraction (on the right in Figure 7.6 and Figure 7.7):

- Sample scans have a lower DP (more negative Y axis value) compared to the buffer-buffer scan.
- A DP difference in sample versus buffer-buffer is greater (more negative value) for higher protein concentrations.
- Transition areas are larger for higher sample concentrations.
- Replicate scans of the same sample are reproducible and parallel to each other.
- The baseline is typically higher after the transition than before the transition. This is due to the change in heat capacity of the sample with unfolding.

Possible causes of the DP difference between protein and buffer-buffer scans being larger than 1 mcal/min (as shown in Figure 7.8) are:

- a buffer mismatch between protein and buffer
- poorly-mixed samples or buffers (due to insufficient purge-refills)
- a dirty cell
- incorrectly filled cells
- an underfilled cell
- bubbles in a cell, or
- a more concentrated protein



1. Buffer-buffer scans

2. Protein scans (0.2 mg/mL)

Figure 7.8 Raw data thermograms with large DP offset

Data analysis

The MicroCal PEAQ-DSC software automatically performs much of the data analysis for multiple sample scans. If needed, you can adjust the settings described below and save them for reuse on other data sets.

The complexity of data analysis and reporting depends on the objectives of your DSC experiments. For example, when evaluating the stability of a protein in different formulations or comparing the stability of a set of engineered proteins, you may only need to compare apparent T_m , total area of the thermogram, and T_{onset} for rank-order stability. However, when comparing the DSC thermograms of a biosimilar to an innovator biopharmaceutical, or a reference batch of protein to a newly-purified batch, you may need a complete analysis, including fitting to the appropriate unfolding model, as well as a similarity assessment.

Most analysis steps and options are described in Analysis on page 43. Following are some advanced settings for analysis.

Baseline Type

The baseline **Type** defines the integration baseline used for subtraction. The type of baseline you choose can impact the total area, the ΔH for individual transitions, as well as the standard deviation and variance for these parameters. Table 7.1 describes the three baseline types.

**Note:**

Use the same baseline option for all data during comparability or biosimilarity studies.

Table 7.1 Types of baseline

Type of baseline	Description	Recommended use
Spline	Polynomial approximation of the baseline.	Thermograms with more than one transition.
Progress	Each point reflects the extent of progress of the reaction. The position of a baseline point at any temperature in the transition region is determined by the fraction of the total area that has been completed at that temperature.	Thermograms with one transition.
Line	Linear connection of the pre-transition marker and post-transition marker.	Complex thermograms that give poor baseline fits with the other baseline types.

T_{onset}

T_{onset} is where the first transition starts to unfold, typically 5–10°C below the first T_m . Protein structure is more labile and proteins are more subject to degradation where T_{onset} values are closer to ambient temperatures. Use this data as a discriminator in comparative analysis of conformational stability of proteins in response to different buffer and stress conditions.

**Note:**

This parameter is only available if specified when producing reports (see Parameters widget on page 65).

$T_{1/2}$

Width of the transition at one-half the peak height. $T_{1/2}$ suggests cooperativity of unfolding transition, i.e. relative extent of conformational heterogeneity, presence of intermediate unfolding states and overall protein dynamics. Use this data as a discriminator in comparative analysis of conformational stability of proteins in response to different buffer and stress conditions.

ΔC_p

Difference in the heat capacity before and after a transition. See the Help for equations.

References

For more information on DSC, experimental design, and data analysis, refer to your Malvern Panalytical application specialist or visit the knowledge center at www.malvernpanalytical.com.

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