MICROCAL PEAQ-DSC

Biomolecular stability analysis for the regulated environment
INTRODUCTION TO DIFFERENTIAL SCANNING CALORIMETRY (DSC)

WHAT IS DSC?

Differential Scanning Calorimetry (DSC) is a powerful analytical technique which characterizes the thermal stability of proteins and other biomolecules. In solution, these molecules often undergo thermally-induced conformational changes, such as the breaking of non-covalent bonds leading to the unfolding of a protein. These types of structural rearrangement are detectable by DSC, as they require the absorption of energy in the form of heat. Even in dilute solutions of protein, the new MicroCal PEAQ-DSC systems detect such heat changes and use them to accurately characterize the thermal stability of the molecule under analysis.

A typical DSC thermogram produced as a protein unfolds

Note the multiple peaks and shoulders detected for this multi-domain protein vital stability data which is uniquely accessed via DSC. The parameters that are derived using DSC allow for a quantitative assessment of the stability of the molecule. The peak of a DSC thermogram is the thermal transition midpoint (T_M). T_M is considered a good indication of thermal stability – the higher the value, the more thermally stable the protein. Multi-domain proteins (like antibodies) typically have more than one peak on a DSC thermogram, so more than one T_M can be determined. In addition to the T_M, there are other important parameters that can be used to characterize stability, such as the T_onset and T_1/2. More detailed analysis of the thermogram can also be performed to determine a number of thermodynamic properties including the enthalpy and heat capacity of the denaturation process, allowing for study of the underlying noncovalent interactions involved in the formation of the complex.

As a protein unfolds (denatures), non-covalent bonds break and a new, likely non-functional, structural arrangement is created.

WHY USE DSC?

DSC analysis allows rapid identification of conditions that deliver optimum stability. The streamlined workflow and automated data analysis provided by MicroCal DSC systems accelerate screening of typical formulations and purification conditions, quickly delivering reliable results with minimum hands-on time, and driving productivity in research and development.

MicroCal DSC instruments are found in many laboratory settings including those in the biopharmaceutical & pharmaceutical industries, contract research and manufacturing organizations, government institutions and academia. A choice of two systems meets the diverse requirements of different laboratories.

How can DSC help me?

The information delivered by DSC is ‘gold standard’, label-free stability data which can be used for a variety of purposes:

In biopharmaceutical development & manufacture:
- Predict & maximize a product’s developability & shelf life
- Optimize purification strategies
- Reliably assess biosimilarity and batch-to-batch comparability (e.g. validation of process changes)

In small molecule drug development & manufacture:
- Rapidly rank ligand affinities to protein target
- Characterize a drug’s protein target

In academia:
- Sensitive and reproducible analysis of protein folding and unfolding
- Characterize lipid and detergent micelles
- Measure thermodynamics of nucleic acid transitions
- Research drug delivery mechanisms

Representative DSC thermogram of a monoclonal antibody, with CH2, Fab, and CH3 domains identified. The dotted gray lines are the deconvoluted peaks of each domain transition, with the three T_M (transition midpoint temperatures) indicated.
**MicroCal PEAQ-DSC**

The MicroCal PEAQ-DSC system provides highly sensitive, easy-to-use microcalorimetry that helps reduce the time and cost associated with stability testing and comparability analysis. This is a manual instrument with a cleaning device, which can be upgraded to the automated version upon request. This complete system requires no additional accessories, reagents or consumables.

The integrated software and automated data analysis support the generation of non-subjective, highly reproducible thermal stability data and help deliver compliance with regulatory requirements, increasing productivity in biopharmaceutical research.

**KEY FEATURES:**
- Screens up to 6 samples/8h with unattended operation after sample loading
- Manual system with cleaning device. Upgrade path to MicroCal PEAQ-DSC Automated is available
- Measurement of very tight binding constants, up to $10^{-20} \text{M}^{-1}$
- 21 CFR Part 11- and Annex 11-ready (PEAQ-Compliance software option)
- PEAQ-Performance – validates your instrument to optimize its performance
- PEAQ-Smart (including PEAQ-Finder) – SOP-based operation and data analysis
- PEAQ-Compare – for quantitative comparability (batch-to-batch and biosimilarity) studies
- Network ready - email updates sent during the analysis to keep you informed

**MicroCal PEAQ-DSC Automated**

The MicroCal PEAQ-DSC Automated system delivers high throughput and sensitivity, with low sample consumption, in an integrated platform for increased productivity. All cell filling and cleaning functions are fully automated, for walk-away operation.

Automated data analysis supports the generation of high integrity thermal stability data and delivers compliance with regulatory requirements, whilst allowing easy integration into existing data handling and transfer systems.

**KEY FEATURES:**
- Screens up to 50 samples per day with unattended operation
- Automated cell filling and cleaning
- Standard 96-well plate sample format
- Thermostatically controlled storage of up to 6 plates
- Measurement of very tight binding constants, up to $10^{-20} \text{M}^{-1}$
- 21 CFR Part 11- and Annex 11-ready (PEAQ-Compliance software option)
- PEAQ-Performance – validates your instrument to optimize its performance
- PEAQ-Smart (including PEAQ-Finder) – SOP-based operation and data analysis
- PEAQ-Compare – for quantitative comparability (batch-to-batch and biosimilarity) studies
- Network ready - email updates sent during the analysis to keep you informed
PEAQ-COMPLIANCE

21 CFR Part 11 and Annex 11 Compliance software: DSC for the regulated environment

The all-new MicroCal PEAQ-DSC software is available with an optional 21 CFR Part 11 compliance module. This makes both MicroCal PEAQ-DSC systems perfect for QC-type work, including root cause analysis and in-process measurements.

Malvern has a wealth of experience working in cGxP environments. We understand the need for easy-to-use analytical systems that enable robust method development and transfer, with the support of 21 CFR Part 11 and also Annex 11 compliance tools and full lifecycle documentation.

PEAQ-Compliance enables the control of electronic records via an audit trail and the logging of key events such as the creation and modification of records. Central to the security of this system is the control and traceability of user interaction with the system and software. Both PEAQ-DSC and PEAQ-Compliance software require user log-in credentials, allowing electronic signatures for records. Metadata, including User ID, date and time logging, instrument information and ID, and method parameters, are saved to electronic records, and all copies or modifications of these records retain the original associated metadata.

Access to electronic records is controlled by the Malvern-Access Configurator (MAC) via Microsoft Windows' security system, and security access levels can be configured to suit each organisation's particular requirements.

PEAQ-Compliance software is linked to a specific MicroCal PEAQ-DSC system serial number and requires an activation key.

KEY FEATURES:
• Electronic records from MicroCal PEAQ-DSC include metadata:
  – User ID, date and time, instrument type, method parameters, etc.
• Copy/modified electronic records retain all original metadata
• Electronic records include audit trail and log events
• PEAQ-DSC and PEAQ-Compliance software require user log-in to provide electronic signatures for electronic records
• Access to electronic records is controlled using Malvern Access Configurator (MAC). Security access levels can be configured as required

PEAQ-PERFORMANCE

Keep your system performing perfectly

A further requirement for analysis in a regulated environment is instrument Performance Qualification (PQ), which includes regular checks on the system to ensure that it is performing within defined acceptance criteria.

PEAQ-Performance, part of the MicroCal PEAQ-DSC software, is a new feature specifically designed to simplify these performance checks.

Even if 21 CFR Part 11 compliance is not required for your DSC data, it is useful to frequently perform DSC analysis of a protein with a well-characterized DSC thermogram as a validation of instrument performance. Regular validation increases confidence in the reliability and reproducibility of your data, eliminating the need for analysis replication which can waste precious sample.

PEAQ-Performance automatically ensures that your instrument is performing at an optimum level, which translates directly to excellent, reliable data, enabling you to make decisions with absolute confidence. Performance checks can be incorporated into the experimental sets, and the software automatically analyzes this data, comparing it to expected results and sending an alert if it encounters a failure – giving the user the ability to stop the instrument and avoid loss of time or sample. PEAQ-DSC’s user-friendly guided workflows with embedded help videos empower any level of user to generate high quality data.

KEY FEATURES:
• Automatic system-ready and baseline stability check before starting a run
• Removes subjectivity and simplifies operation
• Saves time and maximizes data utility
• Built-in automated cleaning protocols
PEAQ-SMART

Easy experimental set-up, fast and intuitive data analysis and report generation

PEAQ-Smart provides new and improved control and data analysis for faster experimental design and implementation. Embedded videos are included to give a visual overview of DSC experimental design and also guide maintenance of the system – these help users of every level proceed with confidence.

In addition, a new PEAQ-Smart report designer is included.

An SOP-Builder is built-in, to guide and accelerate experimental set-up and provide the capability to perform rescans and downscanning for reversible denaturation studies.

Finally, PEAQ-Smart contains PEAQ-Finder, which enables the automated Tm determination of multiple thermograms.

PEAQ-Finder offers automatic peak selection, with improved ability to resolve overlapping transitions. This tool resolves even very minor peak shoulders, allowing for non-subjective Tm picking and the identification and analysis of peaks which were previously undetectable. This all results in faster, more accurate and objective DSC data analysis.

KEY FEATURES:
- SOP-Builder
- Automated data analysis
- PEAQ-Finder: Multi-peak ID and analysis – ideal for multi-domain proteins, including antibodies
- Report designer

PEAQ-COMPARE

Directly compare DSC thermograms, between molecules and between batches

DSC has excellent utility as a biophysical and higher order structure (HOS) analysis technique to investigate the comparability of protein samples. This has obvious value when comparing a biosimilar to a reference protein, but it is also very useful in establishing the comparability of batches of the same protein.

For example, when substantial changes are made to the manufacturing process, a comparability exercise must be conducted to evaluate the impact of the change(s) on the product’s critical quality attributes. DSC is commonly used as a HOS biophysical assay to show that the proteins in two compared samples have highly similar DSC profile fingerprints.

MicroCal PEAQ-DSC Automated systems include PEAQ-Compare, a new software tool which performs rapid data analysis and evaluation on a set of DSC thermograms, and provides an objective, quantitative similarity comparison. PEAQ-Compare evaluates several DSC parameters in order to determine and rank-order comparability, and provides detailed information on residual data.

KEY FEATURES:
- Biosimilarity
- Batch-to-batch comparison
- Comparability rank-ordering
### PRODUCT SPECIFICATIONS AT A GLANCE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MicroCal PEAQ-DSC Automated</th>
<th>MicroCal PEAQ-DSC (Manual system)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technology</td>
<td>Differential Scanning Calorimetry</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>Measurement parameters</td>
<td>Temperature midpoint $T_m$</td>
<td>Temperature midpoint $T_m$</td>
</tr>
<tr>
<td></td>
<td>Enthalpy $\Delta H$</td>
<td>Enthalpy $\Delta H$</td>
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<tr>
<td></td>
<td>Heat capacity change $\Delta C_p$</td>
<td>Heat capacity change $\Delta C_p$</td>
</tr>
<tr>
<td>Cell type</td>
<td>Capillary</td>
<td>Capillary</td>
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<tr>
<td>Cell material</td>
<td>Tantalum</td>
<td>Tantalum</td>
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<tr>
<td>Working cell volume</td>
<td>130 μL</td>
<td>130 μL</td>
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<tr>
<td>Sample capacity</td>
<td>288 (6 x 96-well plates)</td>
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<tr>
<td>Sample volume (minimum in well)</td>
<td>325 μL</td>
<td>250 μL (manual filling)</td>
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<td>Typical sample concentration</td>
<td>0.01 mg/mL - 10 mg/mL</td>
<td>0.01 mg/mL - 10 mg/mL</td>
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<td>Sample throughput</td>
<td>≤50 samples / day</td>
<td>≤6 samples / 8h</td>
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<td>Sample storage temperature</td>
<td>4°C - 40°C</td>
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<tr>
<td>Noise</td>
<td>0.05 μCal/°C</td>
<td>0.05 μCal/°C</td>
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<tr>
<td>Baseline repeatability</td>
<td>1 μCal/°C</td>
<td>1 μCal/°C</td>
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<td>Response time</td>
<td>5s</td>
<td>5s</td>
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<td>Measurement repeatability</td>
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<td>Measurement reproducibility</td>
<td>&lt;0.08°C St.Dev. $T_m$ and &lt;2% RSD on $\Delta H$</td>
<td>&lt;0.08°C St.Dev. $T_m$ and &lt;2% RSD on $\Delta H$</td>
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<td>System reproducibility</td>
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<td>&lt;0.1°C St.Dev. $T_m$ and &lt;5% RSD on $\Delta H$</td>
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<td>Multiple feedback modes</td>
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<td>Yes (passive, high gain, low gain)</td>
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<td>Temperature range</td>
<td>2°C to 130°C</td>
<td>2°C to 130°C</td>
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<td>Maximum scan rate</td>
<td>240°C/h</td>
<td>240°C/h</td>
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<td>Reverse scanning</td>
<td>Yes</td>
<td>Yes</td>
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<td>Cleaning routines</td>
<td>3 pre-programmed routines</td>
<td>N/A (manual cleaning device)</td>
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<td>Cleaning solvents</td>
<td>Water and detergent as standard</td>
<td>Water and detergent as standard</td>
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<td>21 CFR Part 11-ready</td>
<td>Yes, with PEAQ-Compliance software option</td>
<td>Yes, with PEAQ-Compliance software option</td>
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<tr>
<td>Network ready</td>
<td>Yes, with email alert capability</td>
<td>Yes, with email alert capability</td>
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<td>Operating temperature</td>
<td>+10°C to +32°C</td>
<td>+10°C to +28°C</td>
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<td>Storage temperature</td>
<td>-20°C to +50°C</td>
<td>-20°C to +50°C</td>
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<td>10% to 70%, non-condensing (10% to 90% for storage)</td>
<td>10% to 70%, non-condensing (10% to 90% for storage)</td>
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<td>IP51</td>
<td>IP51</td>
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<td>Power requirements</td>
<td>100-240V AC; 50/60Hz, 70W (cell), 400W (max, autosampler), PC as supplied</td>
<td>100-240V AC; 50/60Hz, 70W (cell), PC as supplied</td>
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<tr>
<td>Dimensions (W x H x D)</td>
<td>101 cm x 70 cm x 64 cm</td>
<td>20 cm x 19 cm x 44 cm</td>
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<tr>
<td>Weight</td>
<td>Approx. 25 kg</td>
<td>8.2 Kg</td>
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</table>

1Sample dependent; 2Typical results for ribonuclease (RNase) in 50 mM KAc buffer at pH 5.5, at 60°C/h with high feedback; 3Measure of a stable buffer; 4Using ribonuclease (RNase); 5Range may be extended down to -10°C upon request.
WHY CHOOSE MALVERN PANALYTICAL?

We are global leaders in materials characterization, creating superior, customer-focused solutions and services which supply tangible economic impact through chemical, physical and structural analysis.

Our aim is to help you develop better quality products and get them to market faster. Our solutions support excellence in research, and help maximize productivity and process efficiency.

Malvern Panalytical is part of Spectris, the productivity-enhancing instrumentation and control company.

www.spectris.com

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- e-Learning training courses and web seminars
- Sample and application consultancy

www.malvernpanalytical.com/microcal

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