Malvern | Material relationships

LABEL-FREE BINDING ANALYSIS

MICROCALORIMETRY

MICROCAL DSC **SYSTEMS**

UNDERSTANDING BIOMOLECULAR STABILITY

CHARACTERIZE BIOMOLECULE STABILITY QUICKLY WITHOUT LABELS OR PROBES

Differential Scanning Calorimetry (DSC) is a powerful analytical tool for characterizing the stability of proteins and other biomolecules.

DSC directly measures the enthalpy (ΔH) and temperature (Tm) of thermally induced structural transitions in solution.

This information can be used to predict shelf lives, develop purification strategies, and characterize and evaluate protein constructs or other biotherapeutic entities. In addition, it permits rapid ranking of ligand affinities to a protein target in small molecule drug discovery programmes. DSC enables the study of folding and unfolding without labeling or the use of artificial probes, so molecules are studied in their native states. By determining the heat absorbed by the sample as a biomolecule unfolds, DSC provides a measure of its thermostability and an indication of its long-term stability.

The streamlined workflow and automated data analysis afforded by Malvern MicroCal DSC systems helps accelerate screening of typical formulations and purification conditions, delivering reliable results quickly with minimum hands-on time. These sensitive DSC measurements allow rapid identification of conditions that deliver optimum stability.

With options for high throughput automation and unattended operation, and requiring only low sample volumes, MicroCal DSC systems drive productivity in biopharmaceutical research and development.

They also provide the security associated with a product portfolio based on more than 30 years of experience in microcalorimetry. This is supported by thousands of scientific papers that confirm the value of these technologies in research and development

KEY BENEFITS OF MICROCAL DSC SYSTEMS

MicroCal DSC differential scanning microcalorimeters provide fast and accurate determination of transition midpoint (Tm) and other thermodynamic parameters as indicators of thermal stability. They generate a complete thermodynamic profile to understand the factors that affect a biomolecule's conformation and stability.
A choice of systems meets the requirements of different laboratories:

- MicroCal VP-Capillary DSC delivers high throughput and sensitivity, with low sample consumption, in an automated, integrated platform for increased productivity in drug discovery. (Also available in a manual configuration without autosampler)
- MicroCal VP-DSC is a highly sensitive, easy-to-use system that delivers outstanding quality data ideally suited for research and specialized applications

Applications:

Used widely in the life sciences and drug discovery to study the stability of proteins and other biomolecules, with key applications inDrug discovery for:

- Characterizing and selecting the most stable protein or biotherapeutic candidate
- Eliminating those candidates likely to have long-term stability issues
- Optimizing expression, purification and manufacturing conditions
- Rapidly and easily determining the conditions for liquid formulation

Choose a MicroCal DSC to suit your applications

	MicroCal VP-DSC	MicroCal VP-Capillary DSC
Active cell volume	500 µL	130 µL
Typical minimum protein concentrations	0.02 to 0.1 mg/mL	0.2 mg/mL
Maximum scan rate	90 °C/h	240 °C/h
Temperature range	-10 °C to 130 °C	-10 °C to 130 °C
Typical time per scan	60 to 150 min	35 to 55 min (depends on scan rate and temperatures)
Maximum scans per day	4 to 6 (manual) in 8 h	~ 50 (unattended) in 24 h
Automated cell filling and washing	No	Yes
Samples per 96 well plate	Not available	48

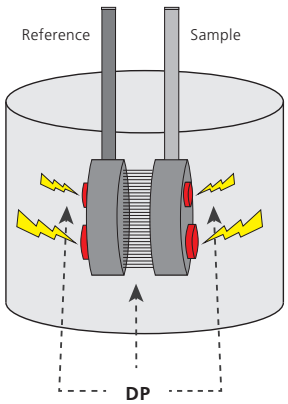
INTRODUCTION TO DIFFERENTIAL SCANNING CALORIMETRY

Differential Scanning Calorimetry (DSC) measures heat changes associated with the thermal denaturation of proteins and other biomolecules, and is used to characterize their stability. A biomolecule in solution is in equilibrium between the native (folded) conformation and its denatured (unfolded) state. DSC measures the heat absorbed when the molecule undergoes 'melting' from its native, biologically active conformation to an unstructured, inactive conformation. The measured thermal transition midpoint (T_m) provides a quick and easy indication of stability, the higher the T_m, the more stable the biomolecule.

Theory into practice

Figure 1.

How do they work?

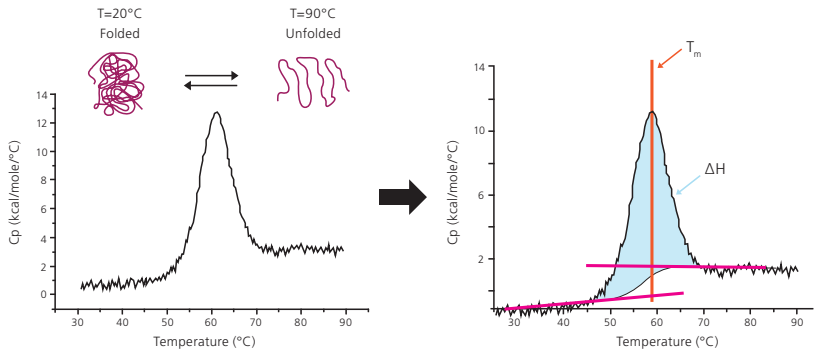


The DP is the differential power added to maintain ΔT~0 between the cells

DP - Differential Power
ΔT - Temperature difference

Figure 2.

DSC experimental principle
Protein unfolding followed through heat changes



T_m is an indicator of stability
ΔH is an indicator of stabilizing forces/energetics

Differential scanning microcalorimeters directly measure the enthalpy (ΔH) and temperature (T_m) of thermally induced structural transitions in biomolecules in solution. Other thermodynamic parameters of unfolding that the data provide include entropy (ΔS), Gibbs free energy and heat capacity changes (ΔCp).

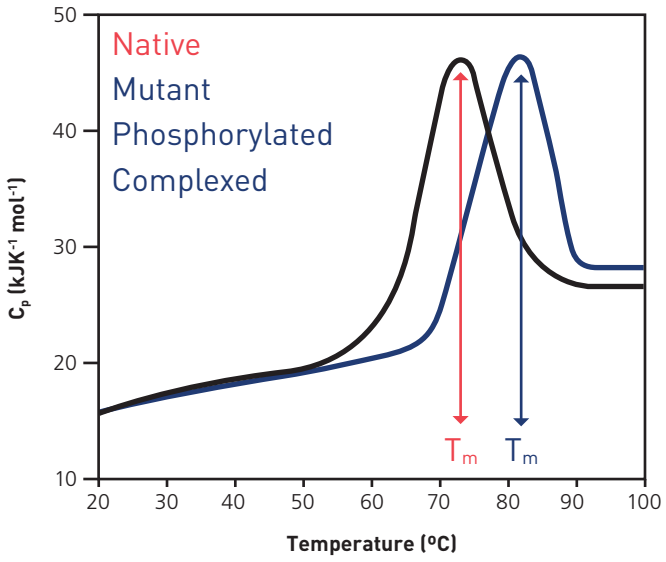
The thermal core of a DSC consists of two cells, a reference and a sample cell, insulated from the surrounding environment. The device is designed to maintain the two cells at the same temperature during the experiment.

A sample in solution is heated at a pre-determined scan rate. Heat absorption when a protein unfolds causes a temperature difference (ΔT) between the cells. DSC monitors the heat change by measuring the differential power that is applied to the cell heaters in order to maintain zero temperature difference between the reference cell and the sample cell. Since protein unfolding, for example, is an endothermic event, it is observed as a positive displacement in the signal (heat capacity). The midpoint of this 'melting' transition is the T_m and the area under the curve is the enthalpy (ΔH) of the process. T_m is an indicator of stability, and represents the temperature at which both the folded and unfolded states are equal and at equilibrium (50% folded:50% unfolded).

Anything that stabilizes the conformation of a protein will cause the protein to unfold at a higher temperature. Anything that destabilizes a protein will cause it to unfold at a lower temperature.

Theory into practice

Figure 3.



Delivered by MicroCal

High sensitivity, high throughput screening with minimal hands-on effort.

- Study protein and other biomolecule unfolding with no labels or artificial probes
- Get results quickly with little assay development, to rapidly identify conditions for optimum stability
- Investigate precious samples, with typical experiments requiring concentrations of just 0.2 mg/ml
- Automate for maximum efficiency, productivity and reproducibility, screening up to 50 samples per day, with application tailored software to streamline workflow and minimize bottlenecks.

A shift in T_m, as Figure 3 illustrates, can be caused by, for example: mutation, post-translational changes such as phosphorylation, or complex formation.

Studying the Tm shift over a range of different ligand concentrations provides data that can be used to calculate the affinity of the interaction. An increase in T_m is associated with an increase in stability. Stabilizing or destabilizing events arise from two different sources: extrinsic and intrinsic. Intrinsic factors are events that occur within the molecule, such as truncation of the molecule, mutation or the addition of an R-group. Extrinsic factors are those that are external to a molecule. Solution conditions are significant here with pH, excipients, and preservatives all having the capacity to affect stability.

The power of DSC

Label-free in-solution measurement provides the ability to study molecules in their native states.

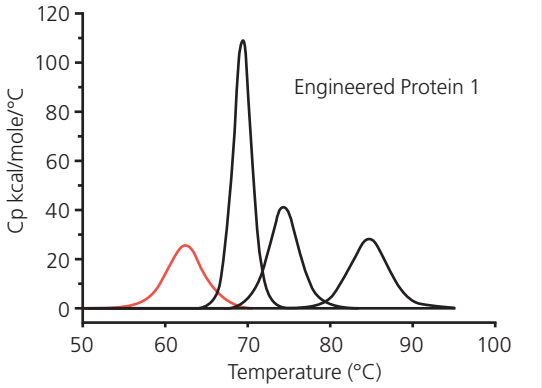
Suitable for a very broad range of applications with a wide variety of solvent and buffers. Ability to analyze high concentration samples - even colored or turbid solutions - with measurements undisturbed by the presence of excipients, makes it ideally suited for real therapeutic formulations. Not limited only to melting temperature (T_m) measurement, but also provides data on the forces involved in biomolecule folding and the mechanisms of unfolding. Easy to use, with no need for labeling or artificial probes, means assay development is minimal, while full automation offers increased laboratory productivity.

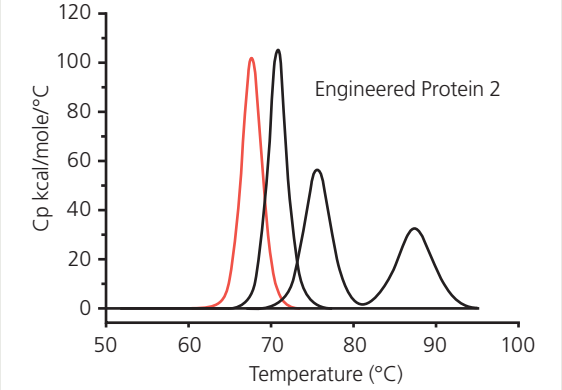
DSC IN ACTION - DEVELOPING STABLE BIOTHERAPEUTICS

Maintaining active, safe and effective biotherapeutics, from development and manufacturing through to targeted delivery in the patient, is a key industry challenge. DSC is a proven and widely-used stability indicating technique. MicroCal DSC systems are integrated into the workflow of pharmaceutical and biotechnology industries, academia and government institutions worldwide. While the following examples provide a snapshot of their use, you can find a wealth of applications information at: **www.malvern.com**

Protein engineering – pick the most stable constructs

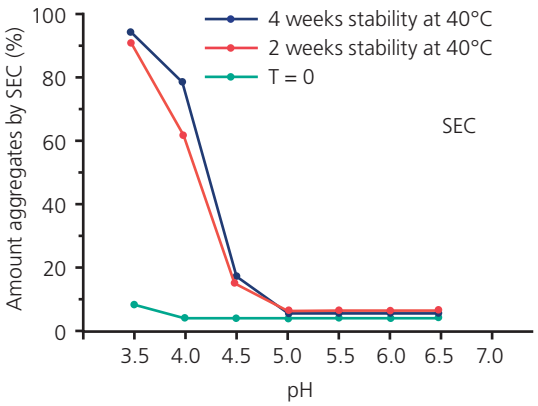
DSC profiling of an engineered antibody showed four distinct domains. An additional modification increased the Tm of one domain (red) and therefore stability.

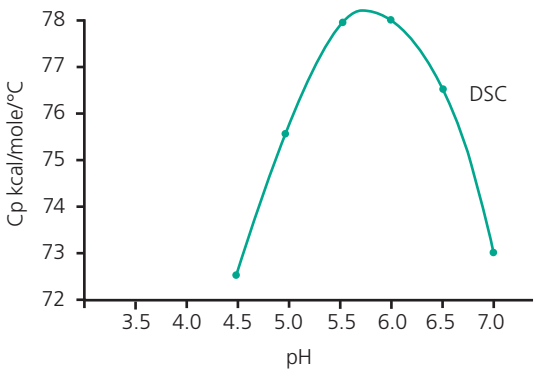




Predict stability early

Early prediction of stability in engineered antibodies allows focus on the most promising candidates. DSC has been used to predict optimal pH conditions (highest T_m) for monoclonal antibodies directly from the initial time zero sample (T0). Other techniques, such as size exclusion chromatography (SEC) – the stability assay method required by regulatory authorities - requires comparison of original samples with samples stored for 2 to 4 weeks. DSC even identified the ≥pH 6.5 values as destabilizing, which would have necessitated an 8 to 12 week study with analysis by SEC.

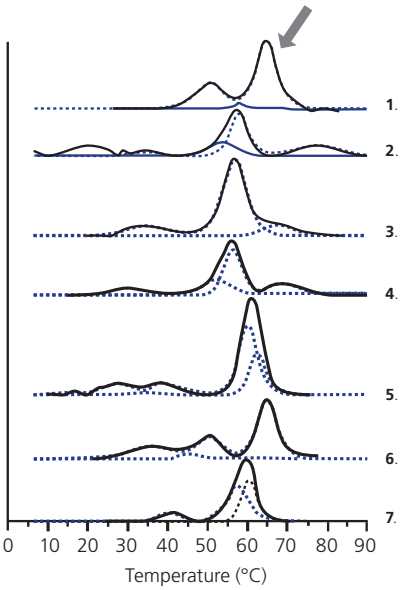




Process development - optimization of purification conditions

Biotherapeutic downstream production processes must achieve high purity and adequate yield on a large scale and within a given economic framework. Using DSC, the most stabilizing loading and elution conditions can be established during process development. In Figure 5, DSC is used to establish the stability of an antibody in a range of buffers.

The antibodies bind at around pH 7 and are eluted at a lower pH. Maintaining antibody stability during elution can be a challenge so identifying the best elution conditions is critical. Using DSC to screen various buffer conditions for a particular antibody showed that mannitol provided the best stabilization and produced a 7-fold increase in yield and a comparable reduction in cost to purify the antibody.

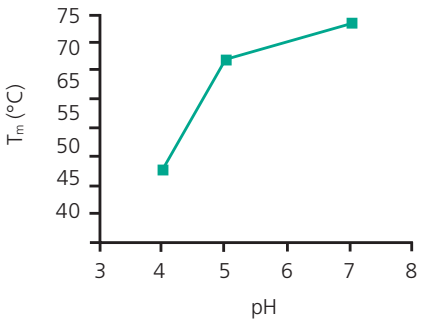


1. Citrate + mannitol, pH 3.5
2. Glycine + Arg, pH 3.0
3. Glycine + Ile, pH 3.0
4. 200mM glycine, pH 3.0
5. 50mM glycine, pH 3.0
6. Glycine + Leu, pH 3.0
7. Citrate, pH 3.5

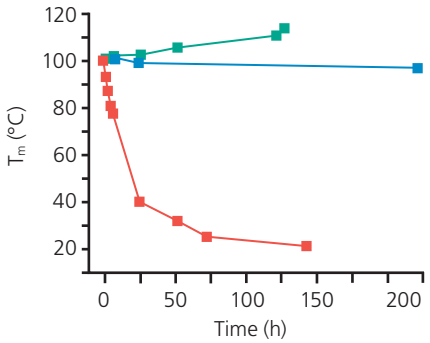
DSC unfolding profiles of an antibody in seven elution buffers. The black lines are the concentration-normalized data and the blue lines are the deconvoluted peaks using the integrated software provided with the instrument.

Stability screening for formulation development

The ability to eliminate destabilizing conditions and focus on the most promising formulations at an early stage is a major advantage of using DSC to examine stability. Real-time stability studies conventionally take several weeks or months to complete and require gram quantities of bulk substances. Samples stored under a variety of conditions are analyzed periodically using methods such as SEC, gel electrophoresis and ELISA. DSC requires analysis only of the initial (T0) samples to characterize thermal stability and predict long-term stability. Each analysis is effectively an accelerated stability study.



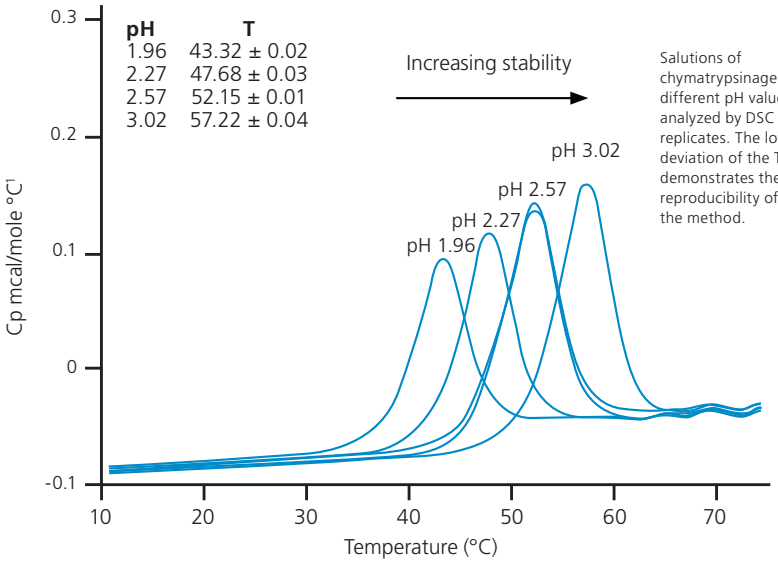
Using DSC, only a few hours work was needed to predict that the protein would be most stable when stored at pH7.



Even after a ten-day accelerated stability study at 37°C. SEC analysis still showed no difference in stability between samples stored at pH5 and pH7.

Reproducible results with high sensitivity and low sample consumption

Boosted by the reproducibility afforded by automation, the MicroCal VP-Capillary DSC system delivers data with extremely low variation, giving you confidence in your results. The high sensitivity of the system takes the pressure off having to produce material in quantity.



Salutions of chymatrypsinagen with different pH values were analyzed by DSC in 5 replicates. The low standard deviation of the Tm values demonstrates the high reproducibility of the method.

MALVERN MICROCAL DSC AT A GLANCE

MicroCal VP-Capillary DSC

The VP-Capillary DSC System is a highly sensitive differential scanning microcalorimeter for characterizing the stability of proteins and other biomolecules. It has an active cell volume of 130 µL, allowing thermodynamic measurements of even precious samples and typically requiring sample concentrations of around 0.2 mg/mL.

For high throughput work, a fully integrated autosampler enables running of up to 50 samples per day.

All filling, injection, and cell cleaning functions are fully automated for walk-away operation.

FEATURES:

- Provides insights into mechanisms of biomolecule unfolding and refolding
- Standard 96-well plate format for high capacity and ease of loading ease
- Minimal assay development needed
- Study molecules in their native state without labeling. Can be used with solutions that interfere with optical measurements
- Industry-proven stability-indicating technique
- Nonreactive Tantalum 61 cells for excellent chemical resistance and to ensure inertness when working with proteins and other biomolecules
- Direct measurement of biomolecular stability in solution
- Screen up to 50 samples/day with unattended operation
- Matched capillary cells provide fast scan rates and fast temperature equilibration.

MicroCal VP-DSC

The MicroCal VP-DSC is a highly sensitive, easy-to-use differential scanning microcalorimeter for the study of samples in solution. Providing fast, accurate transition midpoint (T_m) determination, the MicroCal VP-DSC revolutionized the study of liquid biopharmaceutical formulations by reducing the time and cost of stability testing. In addition, a complete thermodynamic profile is generated to understand the factors that affect conformation and stability.

The VP-DSC is controlled by an intelligent user interface (VPViewer software) and data analysis is performed with MicroCal-enabled Origin software, a leading data analysis package.

FEATURES:

- Complete system, includes ThermoVac sample preparation and cleaning device. No additional accessories to purchase and no reagents or consumables are required
- Industry-proven stability-indicating technique
- Fixed-in-place cells for reproducible ultrasensitive performance with low maintenance
- Direct measurement of biomolecular stability in solution
- Nonreactive Tantalum 61 cells for excellent chemical resistance and to ensure inertness when working with proteins and other biomolecules
- Minimal assay development needed
- Study molecules in their native state without labeling. Can be used with solutions that interfere with optical methods including turbid or colored solutions or particulate suspensions
- Provides insights into mechanisms of unfolding and refolding

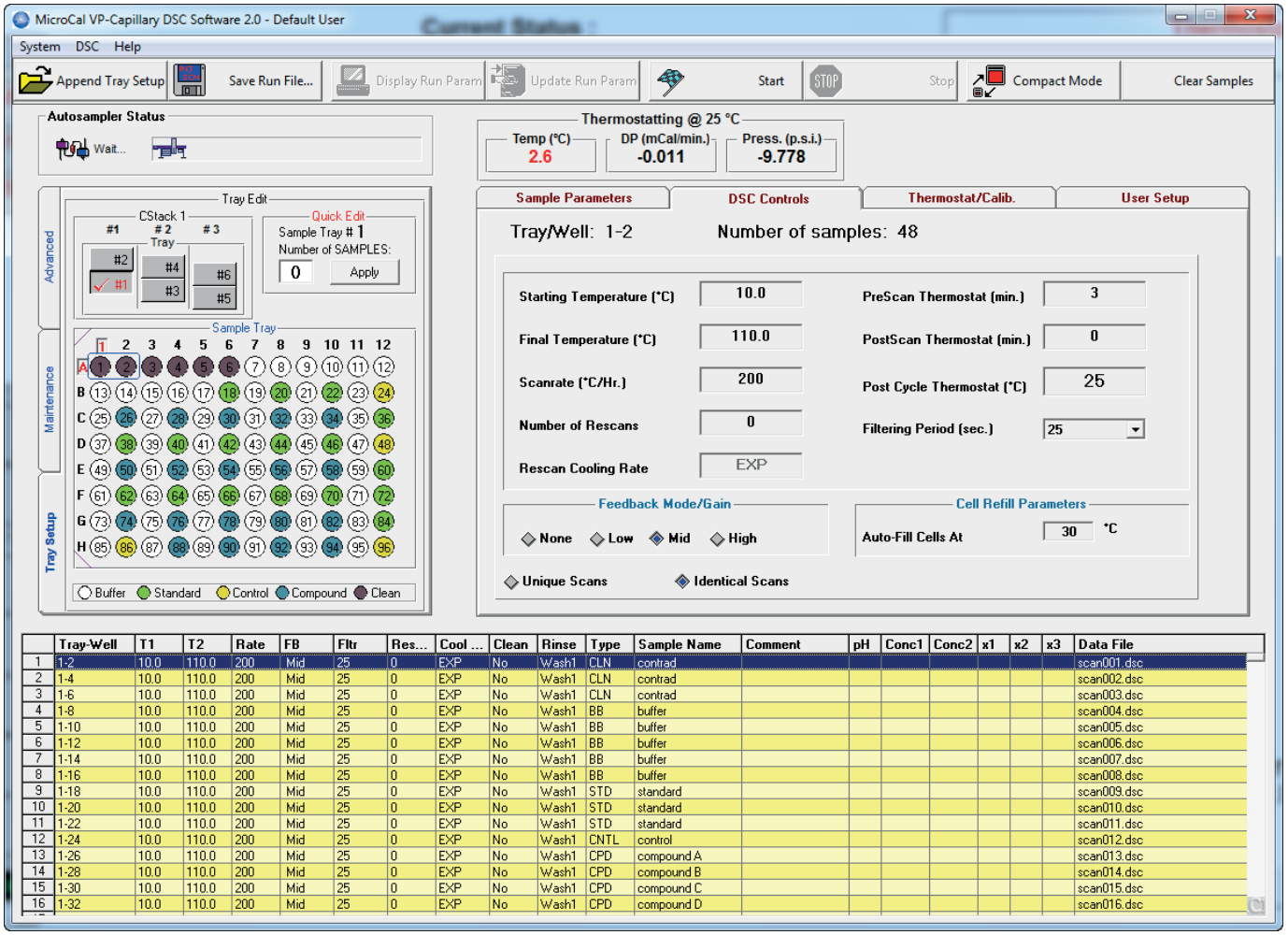
THE MICROCAL VP-CAPILLARY DSC SOFTWARE

MicroCal VP-Capillary DSC Software brings a new level of productivity to automated DSC. Designed to streamline workflow by providing simplified experimental set up it includes, for example, an Excel sample list template that can be saved and re-used rather than rewritten.

The software enables flexible instrument scheduling that is ideal for the multi-user laboratory. In addition, data analysis has been automated and baselines and buffer scans are automatically detected and subtracted, for straightforward analysis. Key parameters such as T_m and T_m onset are determined automatically and the final results are calculated and presented in a summary table.

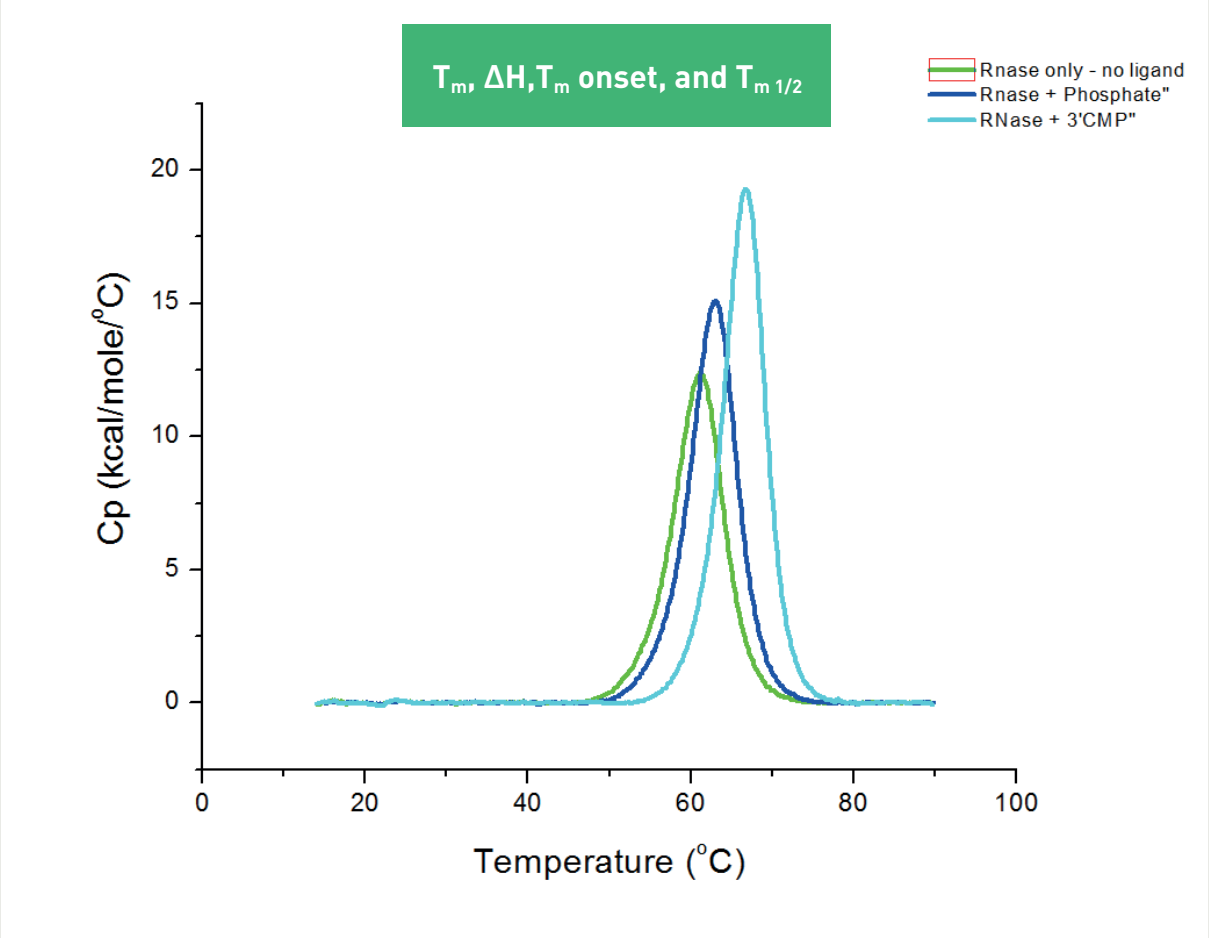
A range of thermodynamic fitting models available in the software supports a variety of applications while improved labelling and sorting functionality further aids data analysis and interpretation.

Easy set up



	Tray-Well	T1	T2	Rate	FB	Fltr	Res...	Cool ...	Clean	Rinse	Type	Sample Name	Comment	pH	Conc1	Conc2	x1	x2	x3	Data File
1	1-2	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	CLN	contrad								scan001.dsc
2	1-4	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	CLN	contrad								scan002.dsc
3	1-6	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	CLN	contrad								scan003.dsc
4	1-8	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	BB	buffer								scan004.dsc
5	1-10	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	BB	buffer								scan005.dsc
6	1-12	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	BB	buffer								scan006.dsc
7	1-14	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	BB	buffer								scan007.dsc
8	1-16	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	BB	buffer								scan008.dsc
9	1-18	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	STD	standard								scan009.dsc
10	1-20	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	STD	standard								scan010.dsc
11	1-22	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	STD	standard								scan011.dsc
12	1-24	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	CNTL	control								scan012.dsc
13	1-26	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	CPD	compound A								scan013.dsc
14	1-28	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	CPD	compound B								scan014.dsc
15	1-30	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	CPD	compound C								scan015.dsc
16	1-32	10.0	110.0	200	Mid	25	0	EXP	No	Wash1	CPD	compound D								scan016.dsc

Scan and sample parameters available in the same window



SummaryTable1

	Sample Name	File Name	Worksheet Name	Sample Type	Buffer	Reference	Tray #	Well #	Conc. 1 mM	Conc. 2 mM	pH	t1/2 °C	DH cal/M	TM Onset °C	Tm 1 °C
1	Baseline	pc2090400	Data1	BB			1	3	0	0		–	–	–	–
2	Baseline	pc2090400	Data2	BB			1	5	0	0		–	–	–	–
3	Rnase only - r	pc2090400	Data3	CNTL	Data2		1	7	0.09	10		7.01	101000	48.99	61.11
4	Rnase + Phos	pc2090400	Data4	STD	Data2		1	9	0.09	10		6.67	117000	51.25	62.81
5	RNase + 3'CM	pc2090400	Data5	STD	Data2		1	11	0.09	10		6.01	134000	56	66.79
6															
7															
8															
9															
10															
11															

SPECIFICATION COMPARISON SUMMARY

	DSC	
Parameter	**VP-Capillary DSC**	**VP DSC**
Measurement parameter	Temperature midpoint T_m	Temperature midpoint T_m
Measurement parameter	Enthalpy ΔH	Enthalpy ΔH
Measurement parameter	Heat capacity change ΔC_p	Heat capacity change ΔC_p
Sample capacity	576 (six 96 well plates)	-
Sample volume	370 µL	700 µL
Cell volume	130 µL	500 µL
Sample presentation	96 well plate	-
Throughput	50 samples / 24 h (automated system)	2-5 samples / 8 h (manual system)
Cell material	Tantalum	Tantalum
Cell configuration	Capillary	Coin-shaped
Noise	0.05 µCal/°C	0.25 µCal/°C
Typical sample concentration	0.1 - 2.0 mg/mL	0.1 - 2.0 mg/mL
Temperature Range	-10 °C to 130 °C	-10 °C to 130 °C
Maximum scan rate	240 °C/h	90 or 120 °C/h
Response time	5 s	7 s
Multiple feedback modes	Yes (passive, high gain, low gain)	Yes (passive, high gain, low gain)
Automated upgrade available	Yes	No
Baseline repeatability	1.5 µCal/°C	1.25 µCal/°C
Cell to cell heat compensation	Power feedback	-
Self contained pressuring system	0 to 45 psi	-
Operating Environment		
- Temperature range	10 °C to 28 °C	10 °C to 28 °C
- Humidity	0% to 70% RH, non condensing	0% to 70% RH, non condensing
Electrical ratings		
- Voltage	100 - 240 V	100 - 240 V
- Frequency	50/60 Hz	50/60 Hz
- Power	70 W	70 W
Weight	25 kg	8.2 kg
Dimensions (W x H x D)	101 x 70 x 68 cm (Auto)	20 x 19 x 44 cm

VALIDATION AND SUPPORT

Malvern's materials characterization technology and expertise enables scientists and engineers to understand and control properties of dispersed systems. Malvern's instruments are used to measure particle size, particle shape, zeta potential, molecular weight, size and conformation, rheology and for chemical identification. This information helps accelerate R&D, enhance product quality, optimize process efficiency.

Areas we work in:

- ACADEMIC BIOCHEMICAL RESEARCH
- BIOPHARMACEUTICALS
- FOOD AND DRINK
- ASPHALT
- PHARMACEUTICAL
- COSMETICS AND PERSONAL CARE
- CHEMICALS
- MINING AND MINERALS
- POWER GENERATION
- CEMENT
- METAL POWDERS
- PLASTICS AND POLYMERS
- SURFACE COATINGS
- ELECTRONICS
- CERAMICS
- ADHESIVES AND SEALANTS

Excellence through experience

Many Malvern systems are used in highly regulated environments and product validation and R&D traceability are priorities for our customers. Operating to ISO9001: 2000 with TickIt accreditation for software development, Malvern is a major supplier to the highly demanding pharmaceutical and chemical industries. Malvern's products play pivotal roles in high quality research and manufacturing throughout the world.
As a global supplier we believe we have responsibility to minimise the impact we have on the environment and operate to both ISO14001 and OHSA18001.

Validation

To help our customers comply with the requirements of the Regulatory Authorities, such as the US Food and Drugs Administration (FDA) and the Medicines and Healthcare Products Regulatory Agency (MHRA), Malvern provides a comprehensive range of validation tools.

These aids follow a user's validation process through from Installation and Operational Qualification (IQ/OQ) to the maintenance phase with annual OQ renewals and the provision of standards for Performance Qualification (PQ). For products subject to FDA regulation, we have solutions to help with 21 CFR Part 11 compliance.

World-class service and support

Malvern offers professional support at all levels. Our intention is to increase your laboratory's productivity through the creation of a working relationship for the lifetime of your instrument providing service support, training and information.

- Global network of fully trained service personnel
- World-wide co-ordination for multi-national companies
- Technical support from the Malvern Helpdesk via telephone or email
- Range of maintenance contracts and service agreements to cover all requirements
- Validation support
- Consultancy-based on site training courses
- e-Learning training courses via the internet
- Classroom training courses
- Web Seminars
- Sample and application consultancy.

No other company offers more

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

Tel +44 1684 892456
Fax +44 1684 892789

www.malvern.com

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