

Microcal PEAQ-DSC Automated

Differential scanning calorimetry (DSC)

DSC calorimeter measures heat changes that occur in the sample (typically a solution of studied biomolecule) during a controlled increase or decrease of temperature, on the basis of a temperature difference between the sample and the reference material. It is a valuable technique for the study of samples in solution providing fast and accurate determination of the transition midpoint T_m – a temperature when 50% of the biomolecule are unfolded. In addition, a complete thermodynamic profile is generated to understand the factors that affect conformation and stability. DSC is a sensitive, easy-to-use technique that requires no assay development, labelling or immobilization.

■ DSC can be used for:

- Determination of the transition midpoint T_m , enthalpy (ΔH) of unfolding due to heat denaturation and the change in the heat capacity (ΔC_p) of denaturation
- Characterization of the stability of proteins or other biomolecules, for elucidation the factors that contribute to the folding and stability of native biomolecules, including hydrophobic interactions, hydrogen bonding, conformational entropy, and the physical environment
- Characterization of membranes, lipids, nucleic acids and micellar systems
- Studies of the process of protein oligomerization
- Assessment of the effects of structural change on a molecule's stability – e.g. protein engineering or antibody domain studies
- Identification and analysis of high affinity interactions

■ Technical specifications

Instrument: Microcal PEAQ-DSC Automated (Malvern)



Features:

- Golden standard for label-free sample stability analysis
- Autosampler for up to 6x96 samples in deep-well plates (up to 288 independent experiments in a row) with controlled temperature (4 – 40 °C)
- Automatic cell filling and cleaning – high reproducibility, minimal loading errors
- 130 µl tantalum capillaries for low sample consumption
- Temperature range: 4 – 130 °C, scanrate of 0°C/hr to 240°C/hr
- Reverse scanning possible – analysis of refolding

■ Instrument operation

Assemblies:

- The instrument is equipped with autosampler for up to 6x 96 samples in DeepWell plates. The autosampler is temperature-controlled in the range of °C.

Data evaluation SW:

- MicroCal PEAQ-DSC SW is used for instrument control and data evaluation. Data can be exported for independent analysis by the user.

Operational mode:

The measurement is performed typically by CF staff. In case of high utilization of the technique by a single user, the user can be trained and perform the measurements himself/herself.

Provided services:

- Standard sample analysis: melting temperature T_m , enthalpy (ΔH_{cal} , ΔH_{vH}), heat capacity ΔC_p
- Instrument user training
- Basic DSC data evaluation training
- Consulting/assistance

■ Sample requirements

- Typical applicable range of sample concentrations is 0.01-10 mg/ml but it may vary for specific samples.
- Proper sample preparation is crucial for the successful DSC measurement. Sample should be homogenous (no aggregates present) and degassed (if possible).
- Sample buffer and buffer for filling the reference cell should be exactly the same (dialysis or lyophilization and dissolution in the buffer for DSC is highly recommended). The pH should be checked before the measurement.
- A minimum of 320 μ l of sample and 320 μ l of corresponding buffer is required for a single measurement. Sample is typically not collected after the experiment.
- If the reducing agent is needed in the sample, usage of up to 5 mM β -mercaptoethanol (or TCEP) instead of DTT is recommended.
- Fluoride compounds can cause irreparable damage of the instrument, therefore it is not possible to measure samples containing fluorides.

It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance.

■ Contacts

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Instrument Location:

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