



| Material relationships



LABEL-FREE BINDING ANALYSIS



MICROCALORIMETRY

# MICROCAL ITC SYSTEMS

UNDERSTANDING BIOMOLECULAR INTERACTIONS

# MEASURE MULTIPLE BINDING PARAMETERS IN A SINGLE EXPERIMENT

Isothermal titration microcalorimetry (ITC) is an essential tool in drug discovery and the study and regulation of protein interactions. Having been developed specifically to meet the needs of life scientists working in these fields, Malvern MicroCal ITC calorimeters deliver the exceptional performance and outstanding quality data needed in these application areas.

MicroCal ITC systems directly measure the heat released or absorbed during a biomolecular binding event. The result is a direct, label-free measurement of binding affinity and thermodynamics in a single experiment. They deliver comprehensive information for studying a wide variety of biomolecular interactions.

Offering high sensitivity, a wide affinity range, reduced sample consumption and options for high throughput with walk-away automation, MicroCal ITC microcalorimeters fully meet the demanding requirements of today's research laboratories. They also provide the security associated with a product portfolio based on more than 30 years of experience in microcalorimetry. This is supported by tens of thousands of scientific papers that confirm the value of these technologies in research and development.

## Key benefits of Malvern MicroCal ITC systems

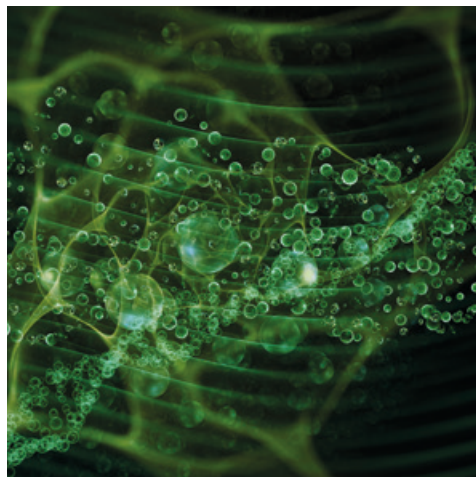
MicroCal ITC isothermal titration calorimeters all allow direct, label-free in solution measurement of binding affinity and thermodynamics in a single experiment, **enabling the accurate determination of binding constants ( $K_D$ ), reaction stoichiometry ( $n$ ), enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ).**

**This provides a complete thermodynamic profile of the molecular interaction. ITC goes beyond binding affinities and can elucidate the mechanisms underlying molecular interactions.**



A range of systems to suit your requirements.

- MicroCal PEAQ-ITC delivers exceptional sensitivity and high quality data with low sample consumption. User-friendly guided workflows with embedded help videos give any level of user the ability to generate high quality data.
- MicroCal PEAQ-ITC Automated combines the high sensitivity of the MicroCal PEAQ-ITC with walkway automation to meet the productivity needs of busy research and drug discovery laboratories.
- MicroCal iTC200 is designed for ease-of-use and exceptional sensitivity. Cell filling, injection and cell cleaning functions are semi-automated and require minimal operator involvement.
- MicroCal VP-ITC is designed for ease-of-use, delivering fast, accurate analysis and outstanding data sensitivity for academic and research environments.



## Applications

Used widely in the life sciences and drug discovery with key applications in:

### Characterizing biomolecular interactions, to:

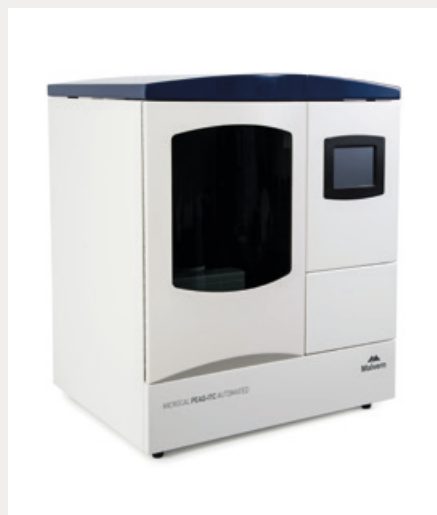
- Confirm binding and activity
- Determine stoichiometry and thermodynamic parameters
- Study structure activity relationships

### Studying the interaction of any two biomolecules including:

- Proteins, nucleic acids, lipids, drugs and inhibitors

### Drug discovery for:

- Hit validation and characterization
- Lead optimization
- Mechanism of action



Photographs not to scale

Model	Sample volume	Sample cell size	Operation	Throughput
MicroCal PEAQ-ITC Automated	370 $\mu$ L	200 $\mu$ L	Fully automated	Up to 42 per 24 h (SIM)
MicroCal PEAQ-ITC and iTC200	280 $\mu$ L	200 $\mu$ L	Manual	8 - 12 per 8 h day
MicroCal VP-ITC	2 mL	1400 $\mu$ L	Manual	4 - 8 per 8 h day

# INTRODUCTION TO ISOTHERMAL TITRATION MICROCALORIMETRY

Isothermal titration microcalorimetry (ITC) measures the binding affinity and thermodynamics of biomolecular interactions, helping to understand why interactions occur. The technique is based on the measurement

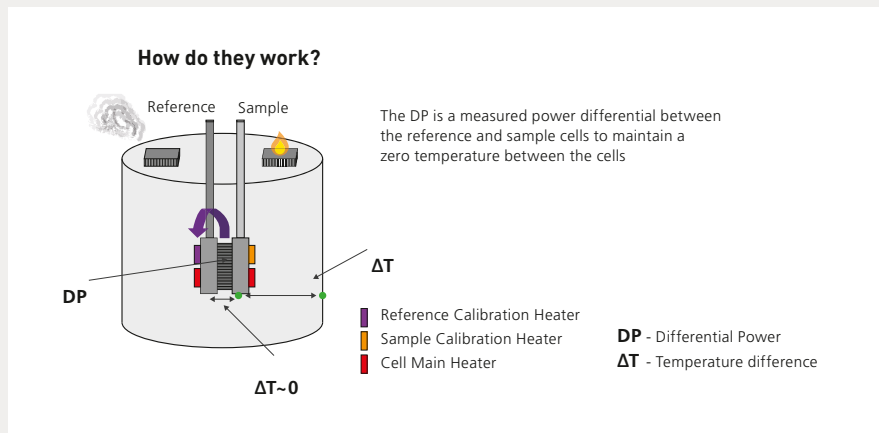
of heat evolved or absorbed when complexes are formed between molecules. It has the advantage of measuring all binding parameters in a single label-free, in-solution experiment, including binding affinity ( $K_D$ ),

reaction stoichiometry ( $n$ ), enthalpy ( $\Delta H$ ), and entropy ( $\Delta S$ ). This reveals thermodynamic data, the forces that drive complex formation, enabling function and mechanism to be described at a molecular level.

## The benefits of ITC

- **Label-free measurement - ensures analysis of unaltered biomolecules in their native state giving a true picture of behavior.**
- **Broad dynamic range - measurement of molecules in solution preserves biological relevance and the sensitivity of the technique accommodates a wide range of affinities.**
- **Information rich data - all relevant parameters – affinity, stoichiometry, enthalpy and entropy – are measured in a single experiment.**
- **Ease of use – quick to first result with minimal assay development, no labelling, no immobilization and no molecular weight limitations.**
- **Broad range of applications - measurements can be made under a wide variety of solvent and buffer conditions.**

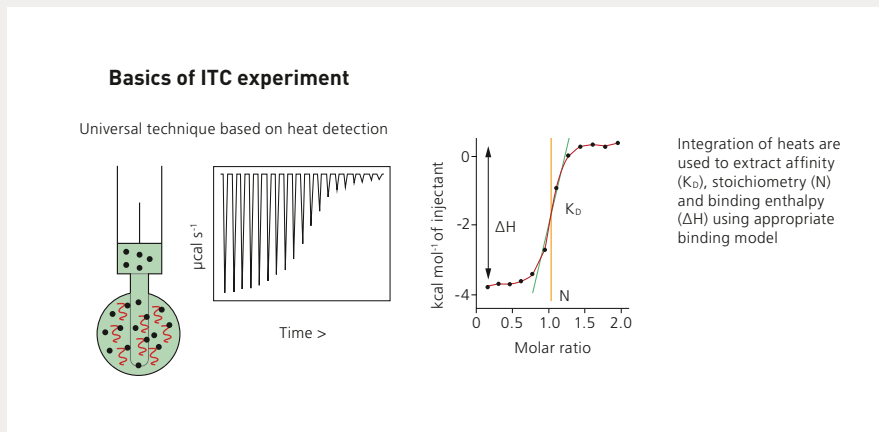
## Theory into practice



Isothermal titration microcalorimeters measure the heat change that occurs when two molecules interact. Heat is released or absorbed as a result of the redistribution and formation of non-covalent bonds when the interacting molecules go from the free to the bound state. ITC monitors these heat changes by measuring the differential power, applied to the cell heaters, required to maintain zero temperature difference between the reference and sample cells as the binding partners are mixed.

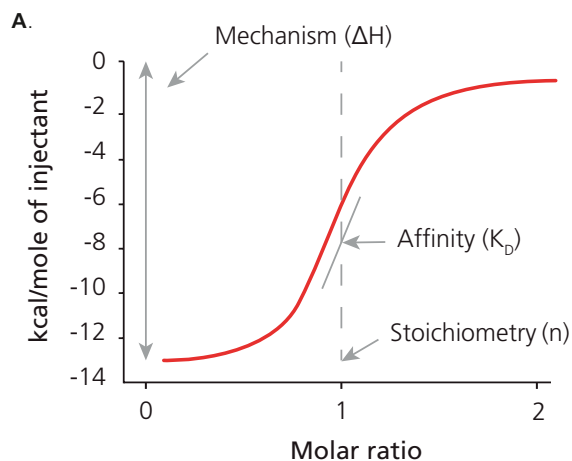
The reference cell usually contains water, while the sample cell contains one of the binding partners (the sample, often but not necessarily a macromolecule) and a stirring syringe which holds the other binding partner (the ligand).

The ligand is injected into the sample cell, typically in 0.5 to 2  $\mu\text{L}$  aliquots, until the ligand concentration is two- to three-fold greater than the sample. Each injection of ligand results in a heat pulse that is integrated with respect to time and normalized for concentration to generate a titration curve of kcal/mol vs molar ratio (ligand/sample). The resulting isotherm is fitted to a binding model to generate the affinity ( $K_D$ ), stoichiometry ( $n$ ) and enthalpy of interaction ( $\Delta H$ ).

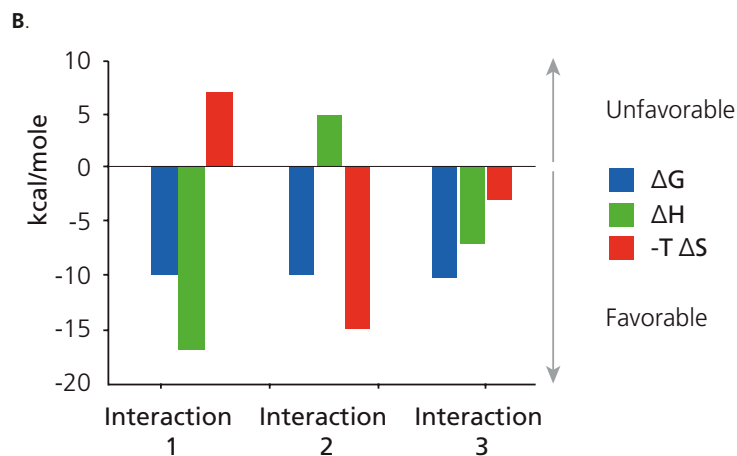


# THE POWER OF ITC

Isothermal titration calorimetry determines thermodynamic properties that tell you why interactions occur. Thermodynamic data reveal the forces that drive complex formation to describe function and mechanism at a molecular level.



**A.** ITC determines thermodynamic properties including: the stoichiometry of the interaction ( $n$ ), the affinity constant ( $K_D$ ), change in enthalpy ( $\Delta H$ ), and change in entropy ( $\Delta S$ ).



**B.** Shown are thermodynamic signatures of three interactions that have the same binding energy ( $\Delta G$ ). The binding energy is related to the affinity. Binding affinity is a combined function of the binding enthalpy ( $\Delta H$ ) and the binding entropy ( $\Delta S$ ). Binding enthalpy reflects the strength of the interaction due to hydrogen bond formation and van der Waals interactions. Binding entropy is a combination of the change in entropy from desolvation and conformational charges upon complex formation.

## Delivered by MicroCal systems

Minimum preparation, maximum results, high productivity

- All binding parameters (affinity, stoichiometry, enthalpy and entropy) in a single experiment
- Measure sub-millimolar to picomolar dissociation constants ( $10^{-2}$  to  $10^{-12}$  M) using direct or competitive binding techniques
- Outstanding sensitivity and data quality gives confidence in results
- Perform a label-free, in solution investigation of any biomolecular interaction using as little as 10  $\mu$ g protein
- Get first results fast with no assay development needed
- Coin shaped cell optimizes sample mixing
- Nonreactive Hastelloy for chemical resistance and compatibility with biological samples
- Compatible with non-aqueous solvents
- Automate for the highest productivity

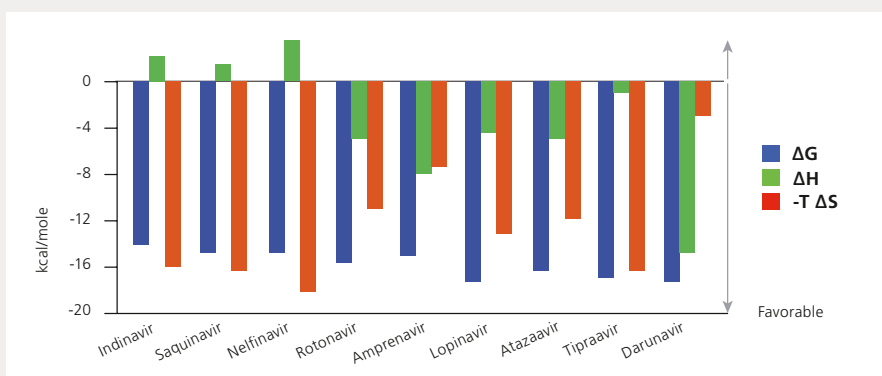
# ITC IN ACTION – PROVEN VERSATILITY

## Theory into practice

Thousands of citations in reference databases illustrate the diverse applications of MicroCal ITC systems. They are used to measure the binding affinity and thermodynamic properties of any biomolecular change that can influence recognition between binding partners.

When combined with structural information, ITC data provide deeper insights into structure-function relationships and the mechanisms of binding. While the following examples provide a snapshot, you can find a wealth of detailed applications information at: [www.malvern.com](http://www.malvern.com)

## Take lead optimization to a new level



Thermodynamic signatures for a complete series of HIV-1 protease inhibitors. The signatures indicate that the most effective and recently developed drugs are more enthalpically driven than the original versions. Data from Freire, *Drug Discov Today*, 2008 October; **13** (19-20): 869-874.

## The value of thermodynamics

ITC permits a multidimensional approach where the contribution of enthalpy and entropy to affinity can indicate favorable chemical modifications to design better drugs, faster. The example shown compares the thermodynamic signatures of a series of HIV-1 protease inhibitors and shows favorable enthalpy for the more effective drugs.

Second generation HIV-1 protease inhibitors, such as Darunavir, have a higher contribution of enthalpy to the total binding energy than first generation therapeutics, such as Indinavir. The conclusion from this study was that the investigation of the interplay between enthalpy and entropy in structure/activity relationships (SAR) would help design new drugs that bind with higher affinity and selectivity.

## Characterize any bimolecular change that can influence recognition between binding partners

With isothermal titration calorimetry you can:

- Verify target activities prior to screening.
- Resolve binding into affinity, the number of binding sites, enthalpy, and entropy.
- Gain a deeper understanding of binding mechanisms for any biomolecular interaction.

Protein-protein interactions are fundamental to all cellular processes and when they malfunction are often the root cause of disease. CHO and coworkers used ITC to understand the role of disordered regions of the protein in molecular recognition. This was achieved by comparing a wild-type protein containing a disordered region (SEC3-WT) with three evolved variants. The results indicated that disordered regions significantly affected the binding energetics. The authors concluded that this type of ITC study has the potential to improve predictive algorithms for protein-protein interactions

## Deeper insights

Drugs should bind to targets with high affinity and selectivity. Traditionally, lead optimization has been driven by studies of the affinity component.

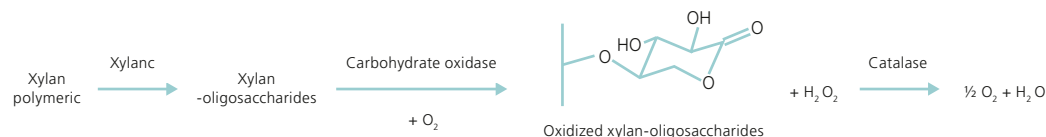
Yet the thermodynamic variables ( $\Delta H$ ,  $\Delta S$ ) are also fundamental to binding and can provide deeper insights into the interactions. MicroCal ITC calorimeters have the sensitivity and throughput for efficient determination of all the binding parameters that can guide lead optimization.



### Optimizing enzyme kinetics with ITC

The kinetics of xylanase is important in bio-bleaching wood pulp and biofuel production. Baumann and coworkers developed a system to measure xylanase kinetics with ITC since the traditional method was complicated and prone to systematic errors. The ITC method was also found to offer greater sensitivity and used less material.

Typically, heat changes associated with xylan hydrolysis are too small to measure directly with ITC. To boost heat flow, an enthalpy amplification system that involved carbohydrate oxidase and catalase was developed. This generated an enthalpy change that allowed measurement by MicroCal iTC200, with signals that corresponded to the amount of mixed xylan oligosaccharides injected.

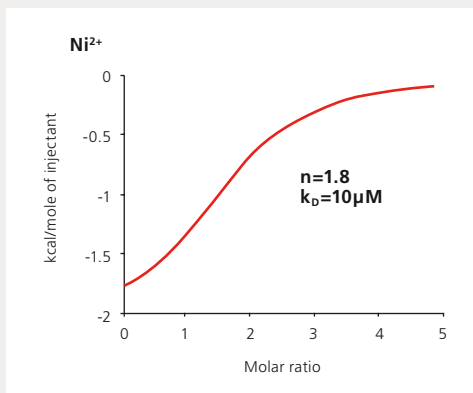
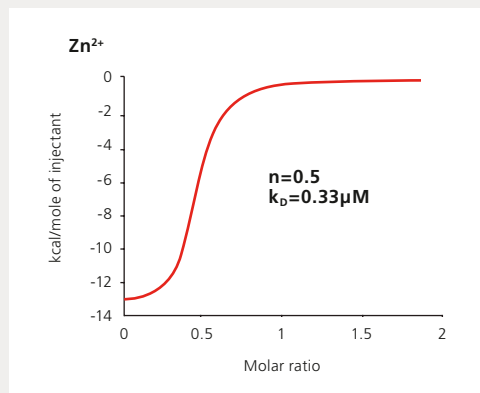


Reaction scheme. (Adapted from Baumann, M.J. et al., *Anal. Biochem.* 2011; **410**:19-26)

### Zinc-induced dimerization of a chaperone

Zambeli and coworkers used ITC to understand the role of disordered regions of UreG, from *Helicobacter*. UreG is a molecular chaperone that activates urease by delivering two nickel ions. The process involves GTP hydrolysis and uses several proteins and metal-ion interactions. Since UreG is unstructured in solution, it has been difficult to understand the structure function relationships.

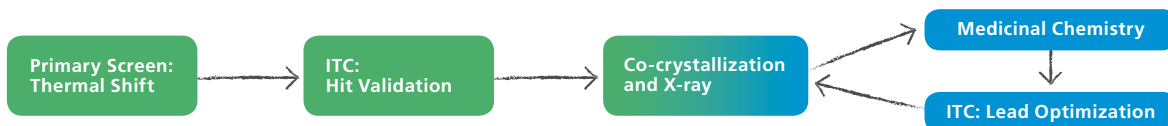
Conflicting evidence suggests that UreG can exist as a monomer or as a dimer. ITC measurements of the stoichiometry indicated that UreG could exist in either form depending on the species of metal ion. Zinc bound to UreG (Affinity 0.33 μM) with two proteins to each ion suggesting that zinc ions cause dimerization. Whereas a close analog, HpUreG, bound nickel with a 20-fold weaker affinity than zinc. The stoichiometry indicated that two nickel ions bound to each protein in process that does not require protein dimerization.



### Predictive power and productivity in fragment-based drug discovery

An automated MicroCal ITC was used to identify and optimize potential lead compounds in an early drug discovery program for treatment of drug resistant tumors. Data from an automated MicroCal ITC was used to validate hits from a primary screen and to accurately rank the affinities of the fragments so that only the strongest binders were selected for co-crystallization attempts and structure based drug discovery program.

The approach successfully predicted which hits would form co-crystal complexes with the target. This was clearly demonstrated, when 12 of the 14 protein complexes chosen from the ITC validation were successfully crystallized. This study underlines that the predictive power of ITC can streamline a fragment based drug discovery (FBDD) workflow and save time.



# MICROCAL ITC RANGE AT A GLANCE

## MicroCal PEAQ-ITC Automated

Combining exceptional performance of the MicroCal PEAQ-ITC with full automation and unattended operation, the MicroCal PEAQ-ITC Automated is a valuable asset for any busy research laboratory.

User-friendly software ensures efficient experimental design while automated data analysis delivers fast, reliable results. The automation and throughput it offers make it a particularly good choice for drug discovery applications such as hit validation where productivity is crucial.

### FEATURES:

- Fully automated with capacity to run four 96-well plates unattended
- Optimized automation scripts for improved performance and assay reliability
- Software that streamlines workflows and improves data analysis consistency for confident decision-making
- Append new experiments 'on the run' to increase productivity
- Single syringe load for multiple titrations (i.e. 4 experiments of 10  $\mu$ L)
- New simplified layout





# MICROCAL ITC RANGE AT A GLANCE

## MicroCal PEAQ-ITC

MicroCal PEAQ-ITC is designed for ease-of-use and exceptional sensitivity. The wide affinity range enables analysis of weak to high affinity binders, with excellent reproducibility. MicroCal PEAQ-ITC analysis software offers experiment design simulation, batch evaluation of large data sets, automated assessment of data quality and a streamlined user interface that guides the user to final figures and presentation quality graphs quickly and easily. MicroCal ITC is an essential tool for any research laboratory studying biomolecular interactions where high sensitivity and fast results are paramount.

### FEATURES:

- User-friendly guided workflows with embedded video tutorials give any level of user the ability to generate high quality data
- High signal to noise gives more confidence in accessing data quality and relevance of generated affinity and thermodynamic parameters
- Automated washing with detergent of the sample cell and titration syringe assists in producing high quality reproducible data
- Analyses all binding parameters (affinity, stoichiometry, enthalpy, entropy) in a single experiment
- Quick to first result with minimal assay development and no labelling
- Sensitive enough to investigate biomolecular interaction using as little as 10 µg protein
- Directly measures millimolar to nanomolar affinities ( $K_D$ ) ( $10^{-2}$  to  $10^{-9}$  M)
- Measures nanomolar to picomolar disassociation constants using competitive binding techniques ( $10^{-9}$  to  $10^{-12}$  M)
- MicroCal PEAQ-ITC analysis software
  - Open multiple experiments in a single session
  - Automated fitting models (One-Site, Two-Site, Sequential, Competitive, Enzyme Kinetics, Dissociation)
  - Automated assessment of data quality
    - Good quality data - Binding
    - Good quality data - No binding
    - Poor quality data - Check data



# MICROCAL ITC RANGE AT A GLANCE

## MicroCal iTC200



The MicroCal iTC200 is designed for ease of use and exceptional sensitivity. Its wide affinity range enables analysis of weak to high affinity binders with excellent reproducibility. Syringe and cell cleaning functions are semi-automated and require minimal operator involvement. User-friendly software guides all operations for fast accurate analysis. MicroCal iTC200 is an essential tool for any research laboratory studying biomolecular interactions where high sensitivity and rapid results are paramount.

### FEATURES:

- Analyses all binding parameters (affinity, stoichiometry, enthalpy, entropy) in a single experiment
- Quick to first result with minimal assay development and no labelling
- Sensitive enough to investigate biomolecular interaction using as little as 10 µg protein
- Directly measures millimolar to nanomolar affinities ( $K_D$ ) ( $10^{-2}$  to  $10^{-9}$  M)
- Measures nanomolar to picomolar disassociation constants using competitive binding techniques ( $10^{-9}$  to  $10^{-12}$  M)

## MicroCal VP-ITC



MicroCal VP-ITC is a sensitive isothermal titration microcalorimeter that is easy to use. Operated via a software interface it delivers fast and accurate analysis. Applications include characterization of the molecular interactions of proteins, antibodies, nucleic acids, lipids and other biomolecules. It is widely used in academia and research laboratories for lead optimization, hit validation, assessing the effect of molecular structure changes on binding mechanisms, and for investigating enzyme kinetics.

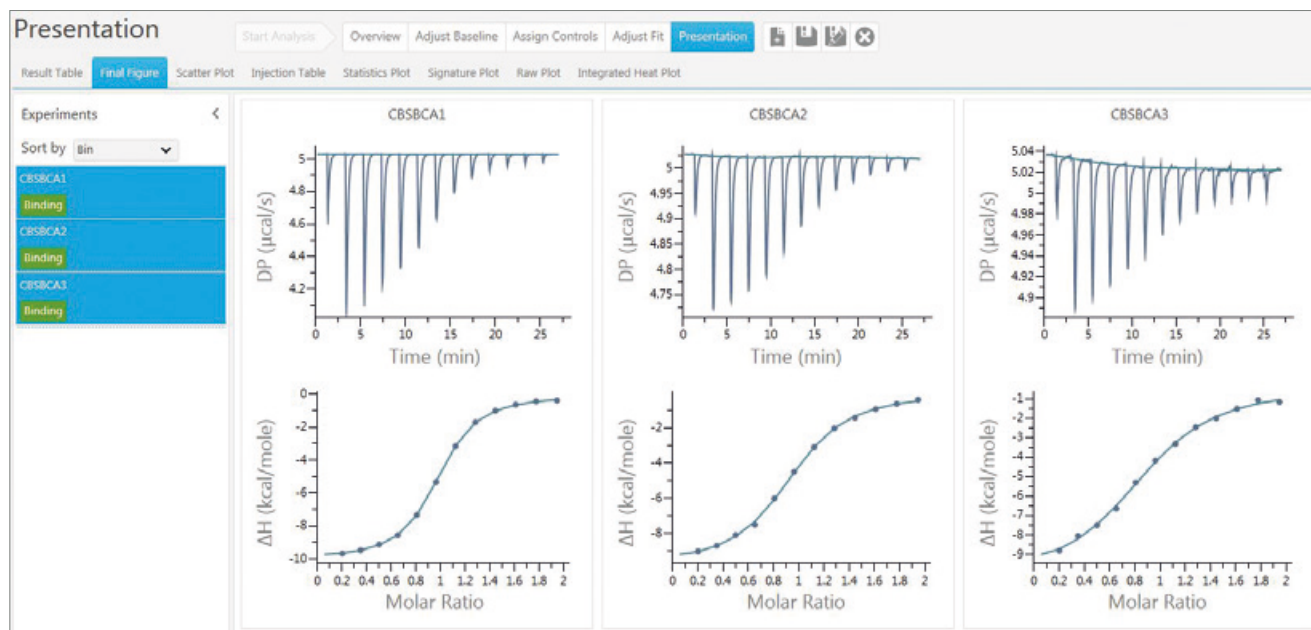
### FEATURES:

- Affinity data provide insights into biomolecular interactions
- The ability to investigate many biomolecular interactions
- Convenience and speed since there is no need for immobilization and labeling
- Free choice of buffers and no limitations with respect to molecular weight
- Unattended operation after sample loading, allowing you to focus on other tasks
- A complete system without the need for additional accessories, reagents or consumables

# USER-FRIENDLY SOFTWARE FOR FAST AND ACCURATE ANALYSIS

## INSTRUMENT CONTROL SOFTWARE THAT TAKES YOU FROM EXPERIMENT TO FINAL RESULTS:

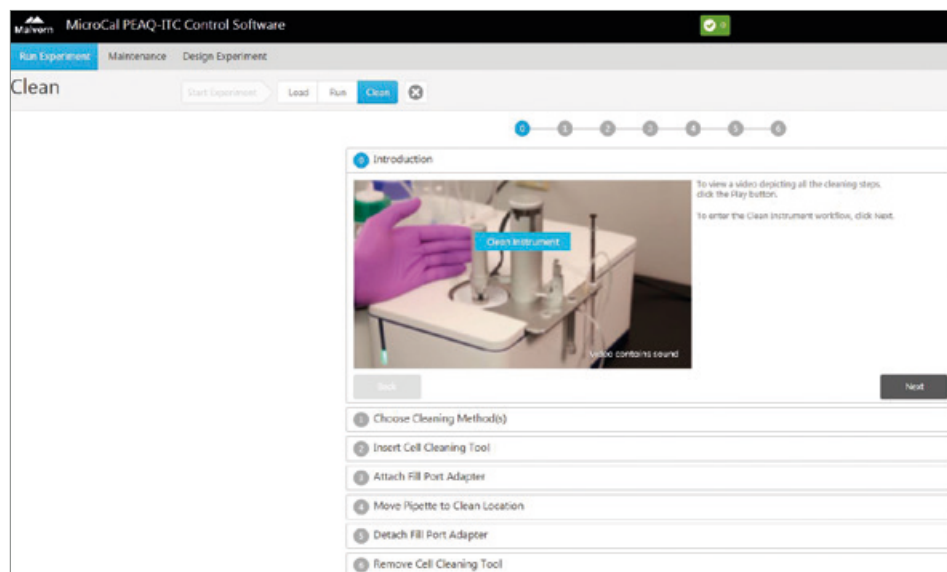
MicroCal PEAQ-ITC instrument control software incorporates all the tools you need to go from experimental design to final results quickly and easily.



## GUIDED WORK-FLOWS

User-friendly guided workflows with embedded help videos give any level of user the ability to generate high quality data with the MicroCal PEAQ-ITC.


## MAINTENANCE HAS NEVER BEEN EASIER



# USER-FRIENDLY SOFTWARE FOR FAST AND ACCURATE ANALYSIS

## EXPERIMENTAL SETUP STEP-BY-STEP

**1 Load Cell**




Move the pipette out of the way (i.e. to the Clean Location).

Fill the loading syringe with 300 µl of sample.

Slowly insert the loading syringe into the sample cell port, gently touch the cell bottom, and move up approximately 1 mm.

**2 Attach Fill Port Adapter**



If the pipette is in the Clean Location, you must press the clamp's release lever.


Move the pipette to the Rest Location.

Align the hole in the pipette's housing to the hole in the pipette's rotating assembly.

Insert the fill port adapter. A soft click should be felt.

Back

**3 Move Pipette to Load Location**



Load approximately 75 µl of titrant in one of the supplied microcentrifuge tubes.


Ensure the microcentrifuge tube has its lid properly situated in the keyed Load Location.

Move the pipette to the Load Location.

Click Next.

Next

**4 Detach Fill Port Adapter**




Move the pipette to the Rest Location.

Detach the fill port adapter from the pipette and return it to its Storage Location.

Click Next.

Back

**5 Move Pipette to Cell**



If the cell is loaded, move the pipette into the cell.

Be sure the pipette is firmly seated in the cell port.

Now you may start your experiment.

Click Done

Back Done

Next

## SPECIFICATION COMPARISON SUMMARY

Parameter	ITC			
	PEAQ-ITC Automated	PEAQ-ITC	iTC200	VP-ITC
Measurement parameter	Affinity ( $K_D$ )	Affinity ( $K_D$ )	Affinity ( $K_D$ )	Affinity ( $K_D$ )
Measurement parameter	Enthalpy $\Delta H$	Enthalpy $\Delta H$	Enthalpy $\Delta H$	Enthalpy $\Delta H$
Measurement parameter	Entropy $\Delta S$	Entropy $\Delta S$	Entropy $\Delta S$	Entropy $\Delta S$
Measurement parameter	Stoichiometry (n)	Stoichiometry (n)	Stoichiometry (n)	Stoichiometry (n)
Sample capacity	384 ( four 96 well plates)	N/A	N/A	N/A
Sample tray temp range	4°C ± 2°C at ambient	N/A	N/A	N/A
Sample volume	370 $\mu$ L	280 $\mu$ L	280 $\mu$ L	2 mL
Cell volume	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L	1400 $\mu$ L
Injection syringe volume	40 $\mu$ L	40 $\mu$ L	40 $\mu$ L	300 $\mu$ L
Injection volume precision	< 1% @ 2 $\mu$ L	< 1% @ 2 $\mu$ L	< 1% @ 2 $\mu$ L	-
Sample presentation	96 well plate	N/A	N/A	N/A
Throughput	Up to 42 per 24 h (SIM)	8-12 per 8 h day	8-12 per 8 h day	4-8 per 8 h day
Cell material	Hastelloy	Hastelloy	Hastelloy	Hastelloy
Cell configuration	Coin-shaped	Coin-shaped	Coin-shaped	Coin-shaped
Noise	0.15 ncal/s	0.15 ncal/s	0.2 ncal/s	0.5 ncal/s
Temperature Range	2°C to 80°C	2°C to 80°C	2°C to 80°C	2°C to 80°C
Temperature stability at 25 °C	± 0.00012°C	± 0.00012°C	± 0.00015°C	-
Response time	8 s*	8 s*	10 s	20 s
Multiple feedback modes	Yes (passive, high gain, low gain)	Yes (passive, high gain, low gain)	Yes (passive, high gain, low gain)	Yes (passive, high gain, low gain)
Automated upgrade available	N/A	Yes	Yes	N/A
<b>Operating Environment</b>				
- Temperature range	10°C to 28°C	10°C to 28°C	10°C to 28°C	10°C to 28°C
- Humidity	0% to 70% RH, non condensing	0% to 70% RH, non condensing	0% to 70% RH, non condensing	0% to 70% RH, non condensing
<b>Electrical ratings</b>				
- Voltage	100 - 240 V	100 - 240 V	100 - 240 V	100 - 240 V
- Frequency	50/60 Hz	50/60 Hz	50/60 Hz	50/60 Hz
- Power	400 W	130 W	70 W	120 W
Weight	91 kg	13.6 kg	9.4 kg	11.5 kg (calorimeter) 9 kg (controller)
Dimensions (W x H x D)	63 x 77 x 35 cm	43 x 46 x 38 cm (calorimeter + wash station)	21 x 34 x 35 cm (calorimeter)	20 x 44 x 37 cm

\*The MicroCal PEAQ-ITC Instrument Response Time is a true time constant. It is the time interval between the first deviation away from the baseline, and the point on the peak that is 63% of the maximum peak height.



# 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 OHS18001.

## 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.

## 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







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### Решения Malvern: Доступность передовых технологий

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