



ISOTHERMAL TITRATION CALORIMETRY

With the Nano ITC, heat effects as small as 100 nanojoules are detectable using one nanomole or less of biopolymer. The Nano ITC uses a solid-state thermoelectric heating and cooling system to precisely control temperature, and a unique removable syringe assembly for efficient and accurate delivery of titrant. The true isothermal power compensation design of the Nano ITC delivers ultra fast response times.

The TA Instruments Nano ITC is engineered specifically for binding and kinetics studies on purified dilute biological samples of limited availability

ITC SPECIFICATIONS



Specs

	Standard Volume	Low Volume
Temperature Range	2 to 80 °C	2 to 80 °C
Temperature Stability	±0.0002 °C @ 25 °C	±0.0002 °C @ 25 °C
Minimum detectable heat	0.1 µJ	0.05 µJ
Maximum detectable heat	5,000 µJ	3,000 µJ
Baseline Stability	±0.02 µW/hr	±0.02 µW/hr
Noise Level	2.5 nW	1.4 nW
Response Time	13 Seconds*	11 Seconds
Cell Volume	1.0 ml	190 µL
Cell Configuration	Fixed-in-place, Cylindrical	Fixed-in-place, Cylindrical
Cell Material	24K Gold* or Hastelloy	24K Gold
Injection Syringe Volumes	100 µL and 250 µL	50 µL

ITC TECHNOLOGY

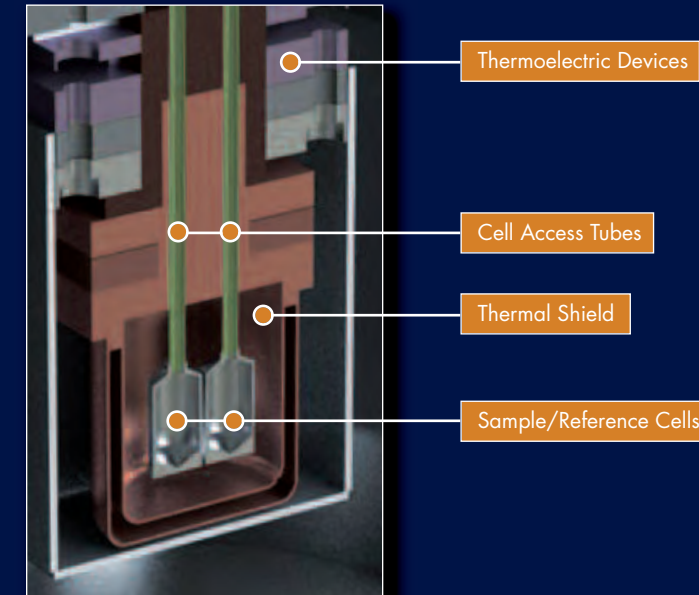
Life Science professionals know that the thermodynamic driving forces of macro-molecular interactions are critical parameters for the design of effective biomedical and pharmaceutical treatments. Calorimetry has become the method of choice for characterizing the thermodynamic driving forces of critical molecular interactions and defining molecular stabilities. Calorimetric analyses are based on accurately measuring the rate of heat absorbed or evolved when the biomolecule of interest interacts specifically or nonspecifically with another macromolecule or ligand (binding studies). The TA Instruments Nano ITC Standard Volume or Nano ITC Low Volume instruments are powerful tools to accurately and efficiently perform these important measurements.

The Nano ITC instruments are designed to improve laboratory productivity and efficiency by performing high-sensitivity analyses on nanomolar quantities of biomolecule. This is accomplished through a combination of a high sensitivity calorimeter, accurate and stable temperature control, and efficient titrant delivery.



The unique removable syringe assembly contains a mechanical paddle stirrer at the end, the speed of which is easily adjusted to accommodate the physical properties of the sample. The integrated titration assembly of the Nano ITC ensures quick-filling, simple cleaning and accurate titrations. The Nano ITC Standard Volume is available with sample cells made from 99.999 % Gold or Hastelloy C to allow for the widest range of reagent chemistry. The Nano ITC Low Volume is available with sample cell cells made from 99.999 % Gold.

The true isothermal power compensation design and the choice of sample cell volumes of the Nano ITC instruments provides the highest sensitivity and flexibility for an ultrasensitive ITC analyzing biological samples in-solution.



The Nano ITC instrument is available in two sample cell sizes. The Nano ITC Standard Volume sample cell volume is 1.0 ml. The Nano ITC Low Volume offers the lowest cell volume at 190 μL , to minimize sample consumption and at the same time provides sensitivity levels over two times better than previously achievable. Heat effects as small as 50 nanojoules are detectable in the Nano ITC Low Volume with a short term noise level of 1.4 nanowatts. Both Nano ITC instruments use cylindrical-shaped cells to make cleaning easy, solid state thermoelectric heating and cooling systems to precisely control temperature, and have the same flexible injection syringe assemblies for efficient and accurate delivery of titrant.

Advantages

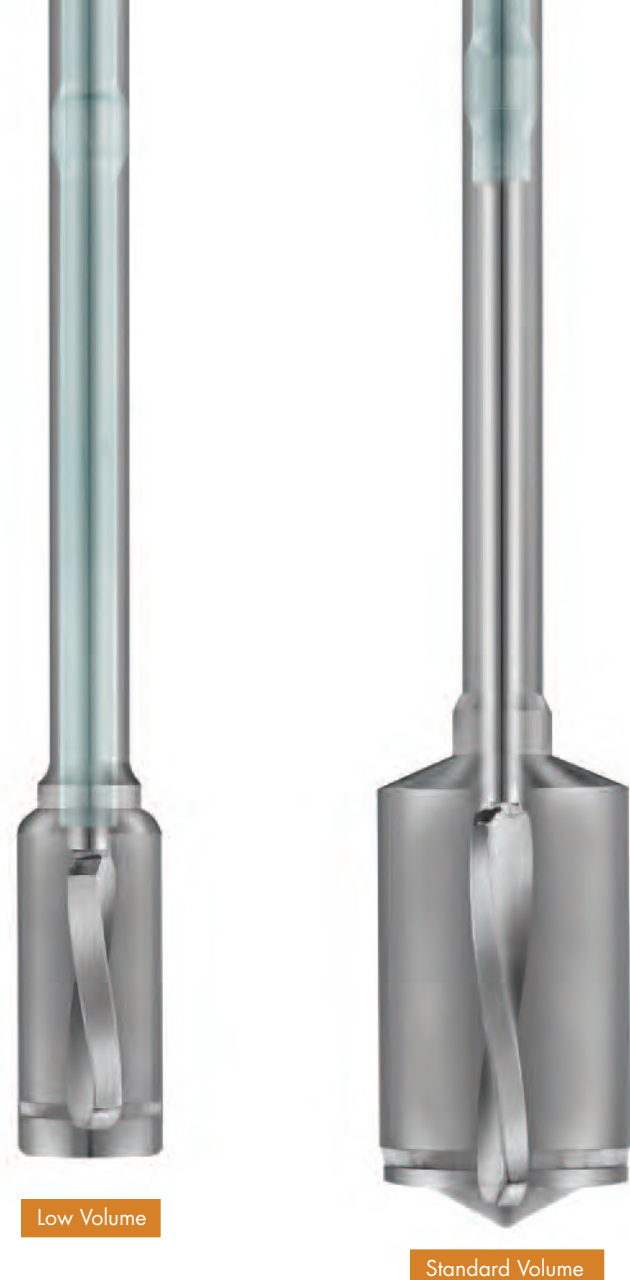
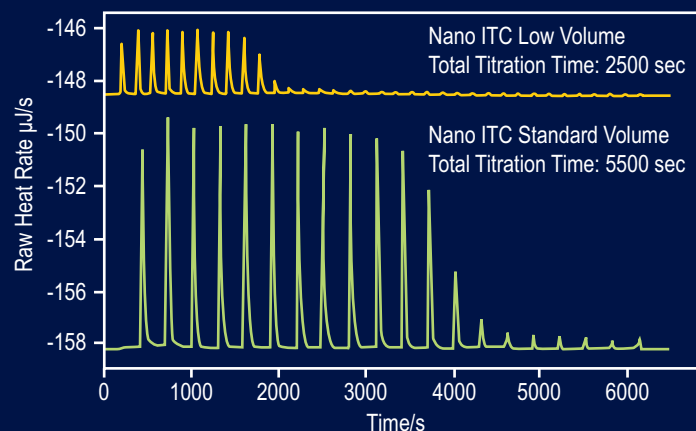
The Nano ITC Low Volume requires substantially less sample and can reduce the time required to complete a titration by one-half. The 2X improvement in sensitivity of the Nano ITC Low Volume ensures that with 80% less sample the instrument will generate accurate and reproducible results.

Nano ITC Low Volume:

Sample Cell = KHCO_3 ; 0.36 mM
 Injection Syringe = HCl; 4.2 mM
 Injection volume = 1.4 μL
 Injection interval = 175 sec

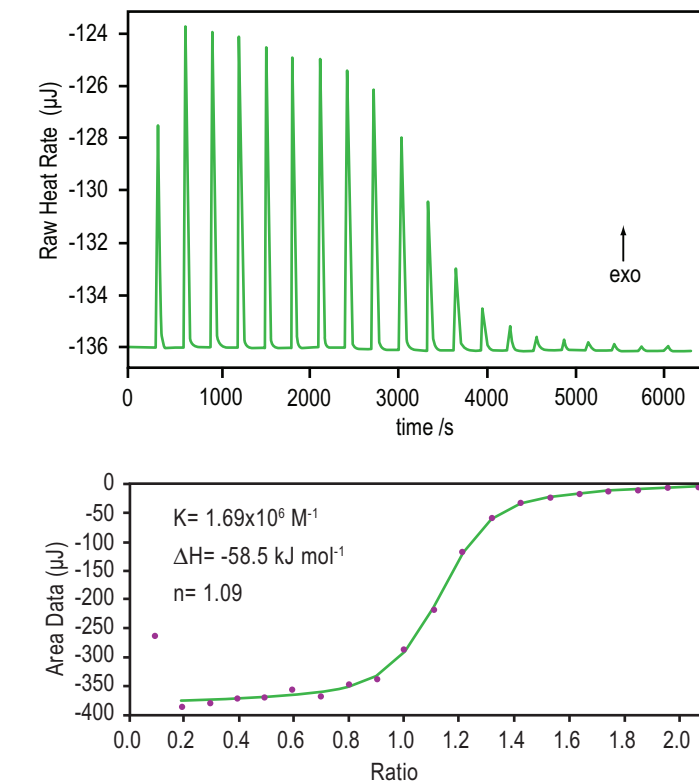
Nano ITC Standard Volume:

Sample Cell = KHCO_3 ; 0.36 mM
 Injection Syringe = HCl; 5.6 mM
 Injection volume = 5 μL
 Injection interval = 300 sec



Characterizing Binding Interactions by ITC

All binding events are accompanied by the evolution or absorption of heat (a change in enthalpy, ΔH). In a single ITC experiment a full thermodynamic characterization of the binding reactions can be obtained. With the appropriate experimental design, fundamental information about the molecular interactions driving the process, as well as the stoichiometry of binding (n) and the binding constant (K_a) is generated. The first figure shows a typical incremental titration (20, 5 μL injections) of an inhibitor, 2'-CMP, titrated into RNase A; $n = 1$, $K_a = 1.69 \times 10^6 \text{ M}^{-1}$, and $\Delta H = -58 \text{ kJ mol}^{-1}$. The second figure shows the same experiment, plotting the individual integrated peak areas vs the ratio of the two binding molecules. As the binding sites become saturated, the amount of heat produced with individual injections decreases. The resulting titration curve reveals valuable information on the enthalpy (ΔH), entropy (ΔS) and overall Gibbs free energy (ΔG) of the reaction taking place in the calorimeter. ITC is a powerful analytical tool and considered the most sensitive assay technique for characterizing the fundamental driving forces of molecular binding reactions.

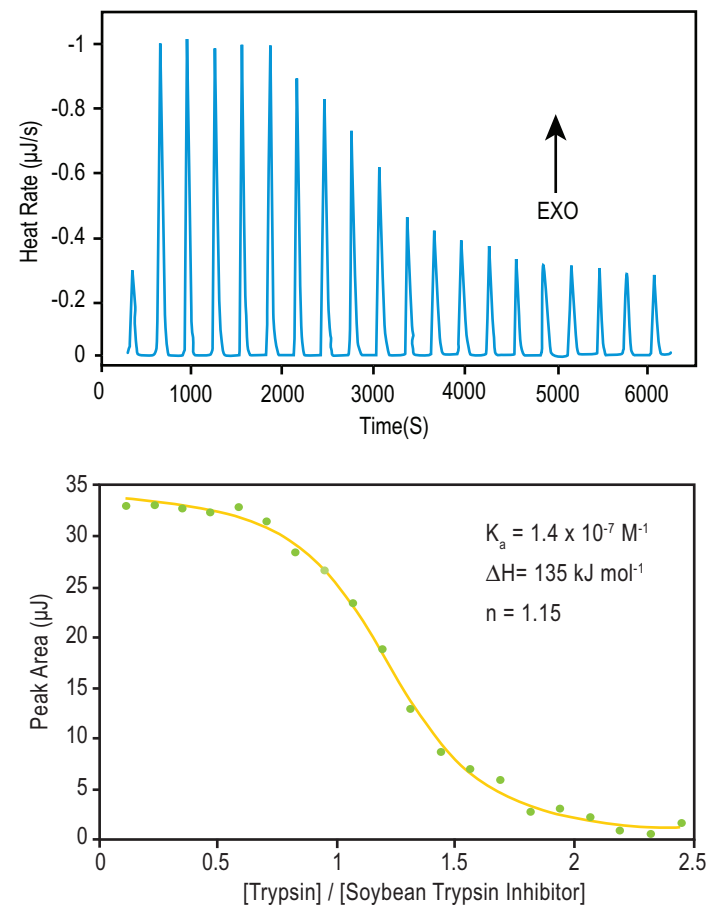


ITC APPLICATIONS

ITC APPLICATIONS

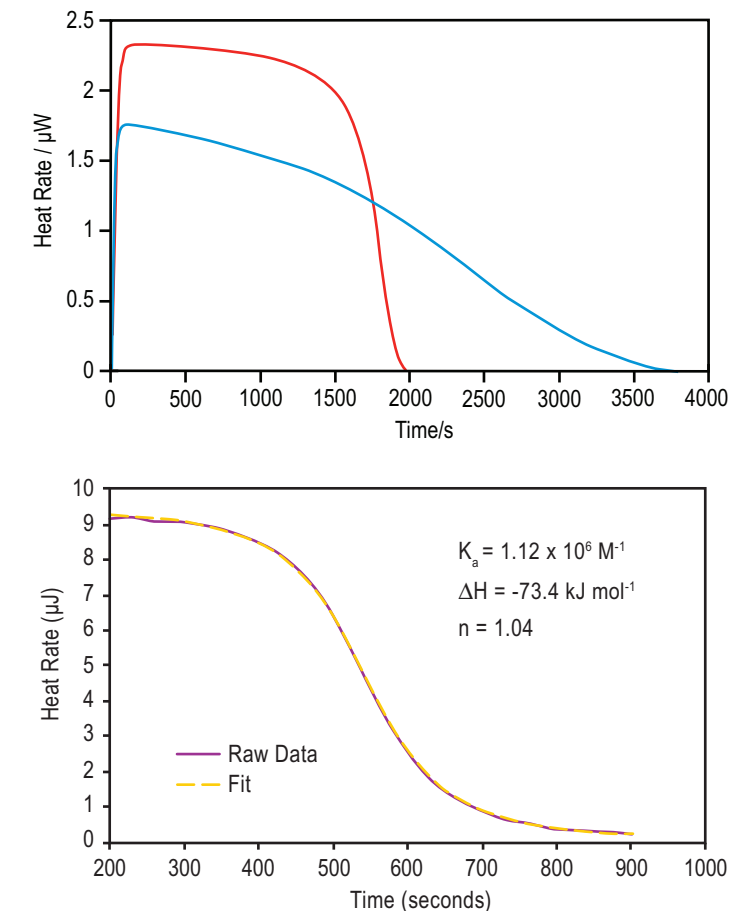
Protein Interactions

When two proteins interact and bind, conformational changes in the proteins, and rearrangement of the solvent in the vicinity of the binding site, result in the absorption or generation of heat. Quantification of this reaction heat by ITC provides a complete thermodynamic description of the binding interaction, the stoichiometry of binding, and the association constant. This figure contains the titration data of porcine pancreatic trypsin into soybean trypsin inhibitor using a Nano ITC. Twenty, 5 μL aliquots of ligand were titrated into the sample cell while the temperature of the system was maintained at 25 $^{\circ}\text{C}$. Top panel: The signal (heat) produced following each addition of protein to the inhibitor. Bottom panel: Integration of the heats over the time course of the experiment; the μJ in each peak are plotted against the mole ratio of the titrant to inhibitor.



Characterization of Enzyme Kinetics

Every reaction generates or absorbs heat, so every reaction can in principle be studied by calorimetry. In practice it has been shown that representative enzymes from every EC classification can be analyzed kinetically using ITC. In addition, ITC analyses are rapid, precise, nondestructive, compatible with both physiological and synthetic substrates, and are as sensitive as spectroscopic techniques but do not require a spectroscopic label or chemical tag. Importantly, ITC analyses of enzyme kinetics are also straightforward. The figure shows the hydrolysis of a single 10 μL injection of trypsin into a solution of BAEE in the absence (blue) and presence (red) of benzamidine, a competitive inhibitor. The area under both curves (representing the total heat output for complete conversion of substrate to product) is the same either in the presence or absence of inhibitor, allowing the K_M and k_{cat} of the reaction under both conditions to be calculated, as well as the inhibition constant.



Continuous Single Injection

Continuous single injection titration is an attractive alternative to the traditional incremental titration ITC for samples exhibiting very rapid binding reactions. These continuous injection experiments can be completed in less total time than normally required for a full set of incremental titrations. This technique provides accurate determinations of stoichiometry (n) and enthalpy (ΔH) for a wide range of binding constants. Continuous injection and incremental injection experiments can be performed in both the Nano ITC standard volume and low volume instruments with no alterations in hardware or software supplied with the instruments.