# Isothermal Titration Calorimetry (ITC) – iTC200

**In a nutshell**: Label-free and immobilization-free studies of biomolecular interactions in solution

**Services**: Biophysics Facility offers ITC calorimeters as open-access instruments. First-time users must complete a short training session before gaining access to the instrument reservation calendar. Training includes performing a test experiment and a complete analysis of the standard binding reaction.

**Location**: Building 50, room 3123

**Description**: ITC is a thermodynamic technique used to determine binding constants and reaction stoichiometry. The ligand solution loaded into the ITC syringe is titrated into the calorimeter cell containing the "macromolecule" solution. As the two components interact, the reaction heat is released or absorbed. The ITC calorimeter directly measures the heat of the interaction between the two reactants and does not require any labeling or modification of the analyzed molecules. Data obtained from a single experiment can provide a complete thermodynamic characterization of the interaction, including not only stoichiometry and equilibrium binding constant ($K_A$), but also the binding enthalpy ($\Delta H$). These values can be used to derive the Gibbs free energy ($\Delta G$) and the entropy ($\Delta S$) of binding. By conducting binding measurements at different temperatures the change in the constant pressure heat capacity upon binding ($\Delta C_p$) can also be obtained. Additionally, measurements in different buffers can provide information about the protonation effects and the electrostatic contributions to binding.

**Typical applications**:

- Native conditions binding affinity measurement without any labeling or immobilization
- Applicable to a wide range of biological systems, including protein, DNA, RNA, and small-molecule interactions
- ITC is particularly well suited for the study of non-covalent interactions between biopolymers and small ligands, such as nucleotides, metal ions, etc.
- Enzyme kinetics measurements
- Full thermodynamic characterization of binding

**Basic instrument specifications**:

- Affinity range: $10^4$ to $10^9$ $M^{-1}$ (relatively high sample concentrations are required for the analysis of weak interactions).
- Extended binding affinity range accessible in displacement titrations when binding competitors are used
- Temperature range: 2 to 80°C
- Nominal volumes: Cell 200 µL, syringe 40 µL
- Wide range of binding models and global analysis of binding data available in the NitPic/SEDPHAT integration and fitting programs

**Sample requirements and recommended buffers**: When performing ITC experiments, it is very important to extensively dialyze both the syringe and cell solutions in the same buffer to achieve a complete chemical equilibrium between the two solutions. Insufficient dialysis is a main source of the ITC artifacts. PBS or similar buffers are preferred, but ITC can handle a wide variety of buffer conditions. Select reaction buffer that provides high solubility and stability of the sample and use buffer concentration sufficiently high to avoid pH effects. Buffer components prone to creating bubbles (detergents, etc.) should be avoided. Antioxidant can result in an additional noise in the collected data. A precise knowledge of concentrations is crucial for data analysis, and for this reason absorbance spectra of all protein components have to be collected prior to the ITC titration. If feasible, samples should be degassed just before loading into the instrument. Do not degas samples prone to foaming or samples containing volatile components.

**Minimum sample amount**: The minimum starting concentrations for an unknown interaction are: 10 µM macromolecule in the cell and 120 µM ligand in the syringe. This should allow detection of dissociation constant ($K_D = 1/K_A$) values in the 10 nM to 10 µM range, and measurement of $K_D$ values in the 100 nm to 2 µM range. When the binding enthalpy is relatively low, the 10 µM cell concentration may not be sufficient and result in a noisy ITC signal. In this case it is recommended to double the loading concentrations if the material is available.

When the approximate interaction $K_D$ is known, the recommended macromolecule concentration in the ITC cell is 20 to 100 times higher than the $K_D$ value. For the 1:1 binding stoichiometry, the ligand concentration in the titration syringe should be at least 12-15 times higher than the cell concentration, so the ligand has to be highly soluble. Explanation: At the end of the titration the total 40 µL syringe volume will be injected into the 200 µL calorimeter cell, resulting in a 5-times ligand dilution. At this point this final ligand concentration in the cell should optimally be 3-times higher than the concentration of the molecule loaded into the ITC cell. To achieve this the initial ligand concentration in the syringe should be 3x5 = 15 times higher than the loading cell concentration.

Sample Volume: The iTC200 requires approximately 400 µL of the protein solution to load the calorimeter cell and 70 µL of the ligand to load the syringe.

**Consumables**: ITC experiments do not require consumables.