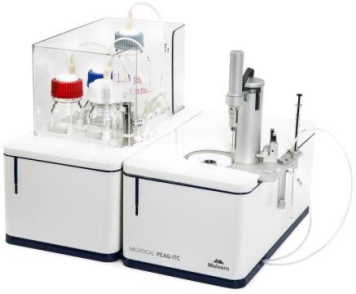
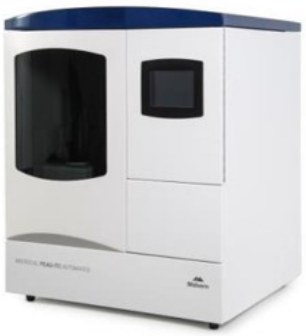
MicroCal™ Isothermal Titration Calorimetry Training

Date: Oct. 5, 2021 **Time: 9:30 AM – 3:00 PM**

9:30 AM – 10:30 AM	Seminar - Introduction to Isothermal Titration Calorimetry Location: Virtual TEAMS meeting • Introduction to ITC instrumentation • Thermodynamics and calorimetry • Applications review
10:30 AM – 11:30 AM	Introduction to PEAQ-ITC instrumentation • Experimental design wizard • Experiment configuration
11:45 AM – 12:15 PM	Lunch Break
12:15 PM – 2:15 PM	Data Analysis
2:30 PM – 3:00 PM	Wrap up/ Basic Troubleshooting





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ITC Sample Preparation Guidelines

Proper sample preparation is essential for successful isothermal titration calorimetry (ITC) testing. The guidelines below must be strictly followed to insure an accurate ITC measurement of stoichiometry (n), heat of binding (ΔH), and dissociation constant (K_D).

The macromolecule solution (the sample to be placed in MicroCal ITC cell): The minimum concentration is 5 μM, if have estimated K_D, use recommended concentrations listed on table below. If K_D is unknown, suggest minimum of 20 μM. Higher concentrations may be needed if ΔH is low. The macromolecule solution MUST be completely dialyzed against desired buffer, or completely desalted with a gel filtration/desalting column.

Estimated K_D	Recommended macromolecule concentration (μM)	Recommended ligand concentration (μM)
1 nanomolar to 0.5 micromolar	10	100
0.5 to 2 micromolar	30	300
2 to 10 micromolar	50	500
10-100 micromolar	30	40 times K_D
More than 100 micromolar	30	20 times K_D

The ligand solution (the sample to be placed in the injection syringe): The optimum ligand concentration is 10-20 times the macromolecule concentration. If have estimated K_D, use recommended concentrations listed on table below. If K_D is unknown, suggest minimum of 100 μM. The ligand must be prepared in exactly the same buffer as the macromolecule.

The sample volumes needed are:

ITC system	Macromolecule volume needed per ITC experiment	Ligand volume needed per experiment
MicroCal ITC200/PEAQ	300 microliters	60 microliters

If ligand requires DMSO for solubility, please contact Malvern scientists for recommended sample preparation and experimental procedure with DMSO.

DTT should be avoided as a reducing agent and replaced by β-mercaptoethanol or TCEP.

Glycerol and other additives, which add viscosity to solution, should be kept to a minimum, recommended final concentration no more than 10% (v/v).

Check the pH of each solution after preparation. If they differ by more than 0.05 pH unit, then one of the solutions must be back-titrated so they are within the limit of 0.05 pH unit. If any particles are visible in either solution, they should be filtered out or microfuged before analysis.

The concentrations of both solutions should be accurately determined after final preparation. Accurate determination of binding parameters is only possible if concentrations of binding components are accurately known.

At least 20 ml of matched buffer must be supplied per ITC sample, to be used for control titrations, rinsing the ITC cells, and sample dilution.