PEAQ-ITC Sample Submission Guideline for
Malvern Isothermal Titration Calorimeter (Auto PEAQ-ITC)

I. Sample Submission

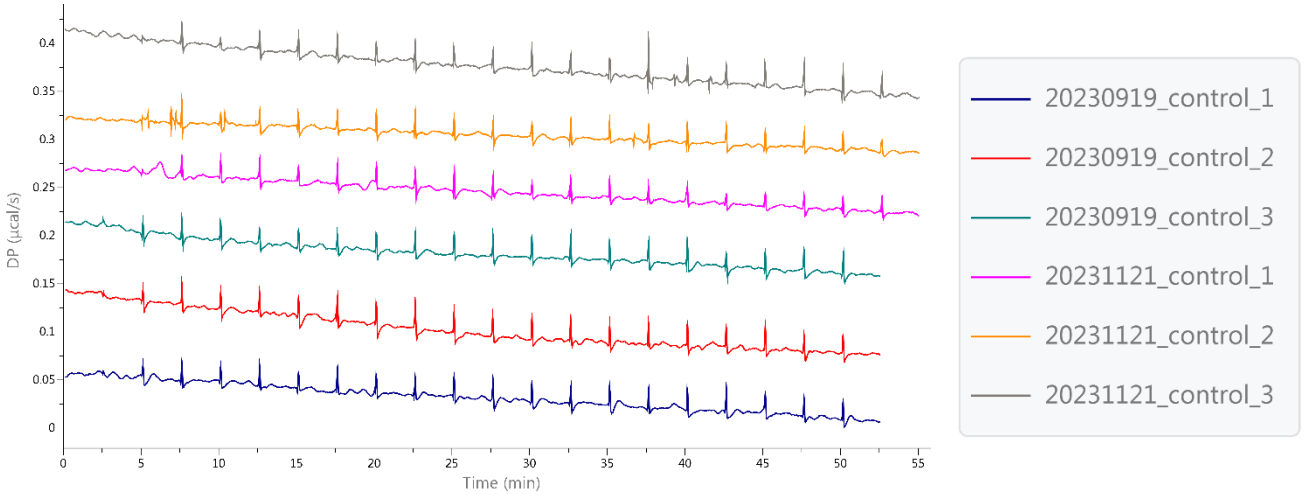
1. Apply an account on the BCF reservation system using your official email address, which will be used to identify your affiliations. BCF will not accept service requests from public e-mail domains, such as gmail, outlook or yahoo, etc.
2. File sample submission form on-line under instrument "Auto PEAQ-ITC".
3. After confirming the charges and experiment time through reservation system, samples and buffers may be submitted to Mr. Xin-Jie Huang (Tel: 27855696 x4024, e-mail: bcf@gate.sinica.edu.tw). If BCF does not receive your samples before the scheduled time listed in the service request application form, the facility will add an extra charge for the delay, unless early notification is sent by emails more than two days in advance.
4. BCF may not compensate for your sample loss or data loss under any circumstances (hardware or software failure, operator error, or others). All experimental results are for research only. Without written permission from Academia Sinica, the user shall not claim, announce, or mislead the public into interpreting that the results of this testing is in any way related to the commercial development of the user. In addition, the user shall not in any form (including but not restricted to commercial marketing, for example advertisements, either online or offline, product packaging, catalogs, investment information etc,) use the title, logo, name, trademark or symbols that are that of Academia Sinica or similar to that of the facility, that gives the false impression of a commercial collaboration.
5. Control experiments of titrant (i.e. ligand in syringe) to buffer and buffer to titrate (i.e. macromolecule in cell) will be counted as experiments and should be listed in the submission form in detail. The control experiment of titrant to buffer is the most important control experiment, which is needed for dilution heat subtraction for data process.

ID	Name	Type	Conc.	Name	Type	Conc.	Temp	Buffer	Note
AZ-1	PBS	Buffer	0	MagicCpd1	Small mol	200	25℃	PBS	Control
AZ-2	ProteinZ	Protein.	20	MagicCpd1	Small mol	200	25℃	PBS	
AZ-3	ProteinZ	Protein.	20	PBS	Buffer	0	25℃	PBS	Control

II. Sample preparation

1. Samples submitted to BCF are non-hazardous, non-toxic and non-pathogenic.
   No radioactive or microbial samples are allowed.
2. Spin samples for 5 minutes at 12,000 g to remove any possible aggregates. Use centrifugal filters if necessary. Check the sample concentrations after centrifugation.
3. Dialysis and gel filtration is recommended for buffer exchange. It is critical that samples in the cell (titrate, i.e. macromolecule) and in the syringe (titrant, i.e. ligand) are in identical buffer. Extraneous heat from buffer mismatch is the major interference of itc experiments.
4. Avoid reducing agents such as dithiothreitol (DTT). If reducing agent is needed, try less than 2mM tris(2-carboxyl)phosphine (TCEP) or β-mercaptoethanol (BME).
5. For one experiment (titration), the volumes required to load the cell and the syringe are **400 µL** and **140 µL**, respectively. In addition, please provide 5 mL extra buffer for rinse.
6. We will mix **20 µL** sample and **4 uL** titrant to check possible precipitation upon titration. The experiments will be cancelled if precipitation occurs during mixing.
7. Use concentration of ***10 to 50 times estimate K_D value*** for samples in the cell and ***10 to 15 times more for the titrant*** in the syringe. For example, for an affinity of 5µM experiment, let alone control experiments, one should prepare 400µL 50-250µM sample in the cell and 140µL 500-2500µM titrant in the syringe. If no estimate K_D can be referred, try **200µM titrant in syringe and 20µM macromolecule in cell** for a start.
8. For the control experiment, prepare another 140 µL of your titrant to be titrated into buffer to check dilution heat and possible buffer mismatch. This will be counted as one experiment and should be listed in the sample submission form.

9. For unknown reasons, the noise of our PEAQ-ITC is higher than before. To reflect the differences, the results of water to water titrations are shown below for your reference. Please contact BCF staff for details before submitting your samples.



Filename	Temp. (°C)	Stir Speed (rpm)	Ref. Power (µcal/s)	Average Heat (µcal)	SD (µcal)
20230919_control_1	25.1	750	5.00 (5.18)	7.51585e-4	7.47283e-2
20230919_control_2	25.2	750	5.00 (5.14)	-2.96639e-3	8.33388e-2
20230919_control_3	25.1	750	5.00 (5.17)	-3.90559e-3	4.71384e-2
20231121_control_1	25.3	750	5.00 (5.07)	-6.29830e-2	0.17168
20231121_control_2	25.2	750	5.00 (5.06)	-1.59202e-2	6.77059e-2
20231121_control_3	25.1	750	5.00 (5.19)	-2.32451e-4	8.43929e-2

III. Experimental setting:

1. The following experimental parameters will be used for most of the samples:
   Cell Temperature (℃): 25
   Tray Temperature (℃): 20
   Stir Speed (rpm.): 750
   Feedback Mode/Gain: High
   Initial Delay (sec.): 300
   Reference Power (µcal/s): 5
   Spacing (sec.): 750
   # of Injections: 20
   Injection Volume (µL): 0.4 for the first and 2.0 for the rest injections
   Injection Duration (sec.): 0.8 for the first and 4 for the rest injections
   Automation Method: “Plates Syringe Clean”, which cleans cell and syringe with 14% Decon90
2. Your samples will be degassed at 20 ℃ under 0.3 atm for 5 minutes before loaded to plates, which are kept at 20 ℃ through experiments.
3. We will first run one buffer to buffer titration to warm up the instrument.
4. Samples will be processed according to the sequence that is filled in the application form; preferably the control experiment goes first.
5. At the end of the experiment, we will perform a buffer to buffer titration.

IV. Data Analysis

1. We will provide raw titration data (power vs. time), integrated heat (by mole of titrant) vs. molar ratio (i.e. titrant/titrate) and control subtracted plots. If no control experiments are submitted, BCF will not provide molar heat analysis, unless clear saturation can be observed at the end of the titration. Plots below show examples of an ITC report.
2. Affinity (K_D), stoichiometry (N) and binding enthalpy (ΔH) may be obtained by fitting to one set of sites binding model. Please refer to Exercise 3 in the **MicroCal PEAQ-ITC Automated System Getting Started Booklet** for evaluation.

3. Please note that data analysis is the responsibility of users. Users are welcome to consult BCF staff for assistance.

V. Acknowledgement

Please acknowledge us if research supported and/or data generated by this instrument results in publications. For example, "We acknowledge Auto PEAQ-ITC data collected by [Operator] in the Biophysics Core Facility funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-CFII-111-201)."