

GatorPlus Sample Submission Guideline

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 the sample submission form in "Service Request" tab under instrument "GatorPlus".
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. Samples submitted to BCF should be non-hazardous, non-toxic and nonpathogenic. No radioactive or microbial samples are allowed.
5. Data analysis is the responsibility of users. However, preliminary analysis is provided in Excel format.
6. BCF will 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.

II. Sample preparation

Samples should be >95% pure by SDS-PAGE or SEC and no visual precipitation when spun at 12,000 x g for 10 minutes. Please provide SDS-PAGE or SEC results by e-mail. If biotinylation is used for immobilization, the ligand buffer must not contain reducing agents (e.g., DTT, β -Me, TCEP), additives with primary amines (e.g., Tris, amino acids, gentamycin, BSA) or solvents with high viscosity, such as glycerol.

Ligand:

Two aliquots of 50 μ L of 1-10 μ M ligand for capturing kinetics assays or one tube of 100 μ L of 20 μ M ligand for biotinylation prior direct-immobilization kinetics assays.

Analyte:

Two aliquots of 20 μ L analyte at a concentration of "**10 x (20 ~100) x Estimate K_D** ", depending on the buffer of your analyte. The concentration may be lower if the analytes are in the same buffer as the assay buffer. If no estimate K_D can be referred, try 10nM for antibodies, 5 μ M for proteins, 50 μ M for small molecules and 500 μ M for polysaccharides and fragments. SA-XT probes and SMAP probes are recommended when analytes are peptides or smaller than 15 kDa.

Buffer:

50mL buffer (same buffer for analytes).

III. Experimental setting:

1. **Immobilization:**

Direct-immobilization: SA probes, SA XT probes and SMAP probes.

Capturing: NTA probes, anti-histidine probes, anti-mouse IgG (Fc) XT probes, anti-human IgG (Fc) Gen II probes, anti-human FAB, anti-GST probes, Strep-Tactin XT probes.

Kinetics assay:

Temperature (°C): 30

Association time (s): 300

Dissociation time (s): 300

Concentration ranges: based on the highest concentration of analyte provided and estimate K_D

Data acquisition Frequency: 5Hz

IV. Data Analysis:

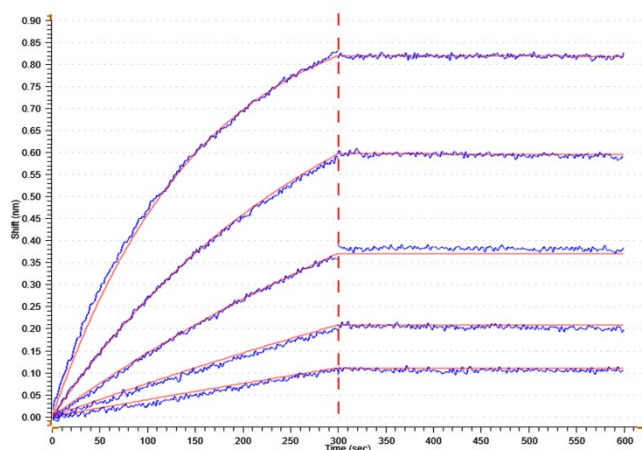
1. Raw data and preliminary analysis can be downloaded through the BCF reservation system.
2. Double reference is performed by subtracting the signal from buffer and the reference channel.
3. The dissociation equilibrium constant (K_D), association rate constant (k_{on} or k_a) and dissociation rate constant (k_{off} or k_d) are presented as is fitted using kinetics "1:1 binding" model in files of Excel format.
4. Please refer to the GatorOne User Manual for additional fitting models, for example, 1:2 binding (bivalent), 2:1 binding (heterogenous ligand) and Mass (mass transfer) models.
5. GatorOne software is available in the laboratory for data analysis.

V. Acknowledgement

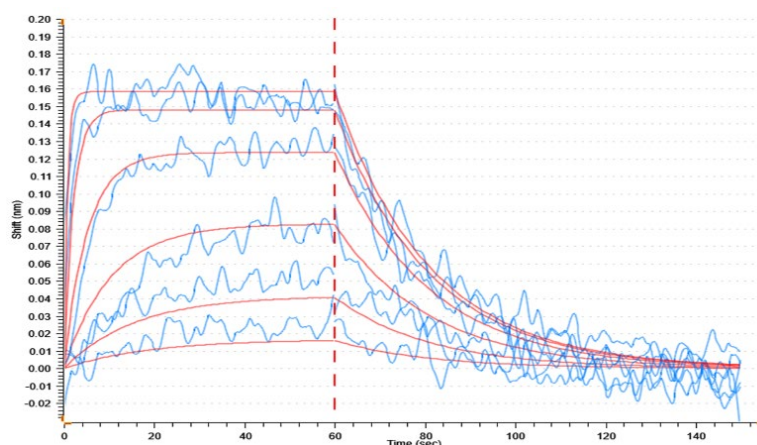
Please acknowledge us if research supported and/or data generated by this instrument results in publications. For example,

"We acknowledge GatorPlus data collected by Xin-Jie Huang in the Biophysics Core Facility funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-CFII-111-201)."

VI. Example of kinetics results.



Conc.(nM)	koff(1/s)	kon(1/Ms)	KD(M)	Rmax	Probe
0.833	6.10E-6	4.92E+5	1.24E-11	0.95	SA
1.667	6.10E-6	4.92E+5	1.24E-11	0.95	SA
3.333	6.10E-6	4.92E+5	1.24E-11	0.95	SA
6.667	6.10E-6	4.92E+5	1.24E-11	0.95	SA
13.333	6.10E-6	4.92E+5	1.24E-11	0.95	SA



Conc.(nM)	koff(1/s)	kon(1/Ms)	KD(M)	Rmax	Probe
123.46	4.78E-2	4.37E+4	1.09E-6	0.16	SMAP
370.37	4.78E-2	4.37E+4	1.09E-6	0.16	SMAP
1111.11	4.78E-2	4.37E+4	1.09E-6	0.16	SMAP
3333.33	4.78E-2	4.37E+4	1.09E-6	0.16	SMAP
10000	4.78E-2	4.37E+4	1.09E-6	0.16	SMAP
30000	4.78E-2	4.37E+4	1.09E-6	0.16	SMAP