Gator Plus (BLI) Start up/Shut down Protocol Simplified

STOP This protocol is for qualified users operating GatorPlus in IBC 402 only. Dr. Jao accepts no responsibility for actions taken as a result of using this protocol. Reading the manufacturer's software user guide is highly recommended.

Sensor pre-wet (rehydration) -> Labeling reaction -> Spin column ->
Ligand loading and blocking -> Kinetic method -> Analyte series dilution

Start up procedure:

1. Turn on control PC. (The instrument is always left on.)
2. Double-click GatorLaunch to initiate the program. Make sure the **Spectrometer Temp.** shows 41 ℃ before starting any experiments.
3. Create a folder in D:\Data\Institute\username\yymmdd to keep the results.
4. Probes should be pre-wetted in your experiment buffer at least 15 minutes in Max plates (250μL per well) for conditioning and equilibration prior to usage.
5. BCF provides NHS-LC-LC-biotin labeling reagent, which is about 0.3μL 10mM aliquot in 100%DMSO for preparing biotinylated proteins. To one aliquot of labelling reagent, add 60μL 50μM of your protein for a one-to-one molar ratio reaction. (example: Use 20μL 150μM BSA per tube, i.e. 20μL 10mg/mL BSA).
6. At the end of the biotinylation reaction, use desalting columns (or spin columns) to get rid of unreacted labeling reagent. Depending on the labeling efficiency, approximate 100 - 500nM of ligand is needed to load SA or SAMP probes (for example, we use 10-50 μg/mL of biotinylated BSA in the BCF M119 training course).
7. Click "Assay Setup" to set up an assay. You may start a simple kinetics assay by importing a template from D:\Data\ATemplates (i.e., BCF Kinetics DR.asy) and modify the parameters. The assay may be saved and exported for future use. **It is advised to design your experiments with double reference subtraction** to check for possible nonspecific binding to the blank (reference) probe survice and to eliminate background that is caused by buffer mismatch or well to well variations. The reference probes (SA or SMAP) may be blocked by loading 1ug/mL biocytin for 60 sec.
8. Remove BCF's green Max plate from the instrument prior any experiments. Fill 250μL buffer per well in your colored Max plate and put it on the right. Fill 180 - 220 μL reagent or buffer per well in the black sample plate and put it on the left. The Max plate may be used for pre-wet and regeneration buffers.
9. Close the instrument lid and click Start at the "Preview" page.
10. Please refer to Gator® Plus User Manual for data analysis.

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Shut down procedure:

11. Take out the Max plate and black plate. Place back BCF's green Max plate.
12. Transfer your result folders to BCF share disk through internet.
13. Delete files shown on the file panel (and the K result in the GatorOne Debug). The result files saved in your default folder will not be deleted by deleting files in the file panel.
14. Turn off control PC and monitor.
20. Leave Gator Plus instrument on.