Biophysical Instrumentation Facility

BLI Notes

Instrument/plates

Reset the machine from Instrument on the top toolbar. (if necessary)

If the Octet gets accidentally turned off only once, the lamp won't need recalibration. After multiple times, it will.

When rebooting if the Octet gets jammed, be sure Windows is open before you turn on the Octet.

Heat range is 29° to 42°.

The big red X on the top toolbar is to stop things if you hear crunching noises.

The Greiner plates are DNase and RNase free and are used for minimal light reflection.

Sensor tips

Pre-wet only the tips you'll use that day. Do it only in the left-hand tray of the Octet because it calibrates them when it picks them up to move them.

Whatever binds to the tips needs to have optical density. Polysaccharides have to be >12 kD to be visible since they aren't dense enough.

PEG 4 or 6 can be used as a linker to give flexibility to your ligand.

There's a biotinylation cheat sheet on the desktop.

Fluorophores will not affect the Octet's optics. If they interfere with your binding, that's bad. With GFP attached to analytes, people have been seeing low binding signal.

Tips can tolerate 0.01% SDS and 1 mM βME. SA tips are okay in 2 M urea at pH 7.5.

Material coming off the tip after loading Ni-NTA tips is no big deal.

HIS tags may not be exposed so put some NaCl in the buffer to reduce electrostatic interactions. Also use salt in buffer when using DNA.

SSA tips need longer than 10 minutes to hydrate.

To regenerate SA tips, see Tech Note 14 or pp. 13-15 of App. Note 14.

APS tips are for hydrophobic work such as membrane proteins.

Amine-reactive tips (AR2G) can be pre-wet in plain water.

Non-specific binding

To prevent non-specific binding, you can use:

> Ovalbumin
> Casein
> Bovine IgG
> DMSO (keep it below 5%)
> PEG
> Gelatin

If the initial binding to the tip goes very fast, that might be non-specific and/or too much ligand. You can limit this with the Threshold setting on the Assay definition tab.

Always include "buffer only" and "analyte only" controls to check for buffer drift and non-specific binding. These are the reference wells and they can be subtracted during data analysis.

Buffers/samples

Keep the buffer the same throughout the experiment plate. If you change buffers within an experiment, you may need to wait longer for the baseline to stabilize.

A tinge of color or fluorescence in a sample won't make a difference but opacity will.

Use one column of buffer in the plate for all buffer steps to prevent inter-step shifts. There's very little carryover of material. (femtomoles) The software rejects multiple buffer columns.

Glycerol is okay in buffer as long as it's the same concentration throughout.

Suggested starting concentration ranges

For loading tips, a range of 1-20 ug/ml is good, up to 100 nM. For association, 10 nM -- 400 nM is good.

A good buffer would be 1X PBS, 1% BSA, 0.05% Tween-20. BSA can be used down to 0.01%.

If you know the K_D of your system, start at a 10X concentration and reduce it step-wise. If you don't know your K_D, just fish around.

If NSB is high, try reducing the loading concentration on the tips.

If association never levels off, load tips to only 1 nm to lower heterogeneity.

Experimental design

Don't analyze data while acquiring!

ELISAs can be transferred to this technology easily. See App. Note 2.

Put lots of information into the acquisition software to make things easier during data analysis.

You want linearity during tip loading so you don't put too much material on. That can cause aggregation or steric hindrance.

If you have tight binders, run the association for a shorter time.

Always include "buffer only" and "analyte only" controls to check for buffer drift and non-specific binding.

If reference sensors are used, you can subtract their NSB value. (a small molecule problem)

Samples are recoverable out of the plate after an assay as long as they've evaporated for only 30-60 minutes.

Changes you make during the experiment (such as truncating a step) won't show up in the data analysis software. You'll have to change things there later.

If you're doing multiple loading steps (to check concentrations), don't uncheck the tip re-rack option.

Kinetics hints

Have all solutions in the same buffer. Use Ni-NTA or SA sensor tips.

You need at least 4 concentrations to get a good K_D.

You need the molarity of your solutions so a K_D can be calculated. (can be added later)

Run dissociation for at least 900 seconds to get a 5% reduction in signal so the software can do accurate calculations. Stay under 3 hr though because of evaporation.

Load the smaller thing onto the tip and bind the bigger one to it. But if binding in one orientation isn't working, try flipping the experiment and put the other binding partner on the tip.

Quantitation hints

Have all solutions in the same buffer.

Use the same isotype of Ab.

Use HIS 2 sensor tips.

The method has to go in the order columns 1-12. It can't back up (like kinetics can).

Software

The User Guides for both the data acquisition and data analysis software are located on the desktops on both computers.

To add or change a concentration in an experiment, under the Analysis tab, R click on the sample and choose Edit Sample/Sensor. Alter the concentration there.

To keep "loading only" data, there's a button above the graph for exporting it.

You can flip data while acquiring it or later during analysis. R click on the first value in the Color column and Flip Data is in the menu which pops up.

If you don't even do a fit, you can save the report and you'll see your raw data graphs.

If you forget to send your data to your folder, it will go to a Temp. folder on the C drive.

Don't leave your experiment name as Experiment_1. Others have used that and your data will mix in with theirs.

If you have only 3 tips in a column and they're not next to each other, the software can't handle that for Sensor Assignment. Just go ahead as if all tips are there and only the real ones will show up in the experiment.

For running more than one assay on a single plate, create the first one then click New Assay and another will replicate itself in a new color.

To subtract only one reference tip at a time, define the two reference sensors on the LEFT plate map, then choose Custom subtraction. Move one reference tip over to the RIGHT plate map, do the analysis, then do the next one separately.

The Custom button also lets you dictate which wells have the reference subtracted.

Data Analysis

R^2 is more important than chi^2. The chi^2 should be < 3. The R^2 should be > 0.95.

You can't average three different runs all together. Afterward, you can average the chi^2 values and residuals.

K_{on} and K_{on} error tell you how good the K_D is. They should be within two orders of magnitude of each other.

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