

Increasing the Signal

The recommended max signal for equilibrium and kinetics measurements should range from 0.5 to 2.0 volts. This Tech Note discusses available options to increase signal as well as some considerations to keep in mind.

Run a Higher CBP Concentration

Signal is directly proportional to the Constant Binding Partner or CBP concentration, so running a higher CBP concentration is the easiest way to get a higher signal. Bear in mind that if the ratio of CBP over K_d becomes too high your measurement is unlikely to resolve an accurate K_d . See Tech Note 220 *Theory curve (TN220) > A Guided Tour -Part 1> Equilibrium Curve* for more information.

If you have no idea what the K_d of the system is, go ahead and run a curve with a higher CBP concentration. Although this curve may be concentration controlled (ie higher ratio), it will serve two important purposes 1) it can define the concentration of the CBP, which may not be fully active so therefore not as far above the K_d as expected, and 2) it can be a high ratio curve in an n-curve analysis. After running the high ratio curve, if the CBP still needs to be lowered, consider the rest of the options listed below. You needn't stick to just one of the approaches described, you can mix and match them as needed.

Increase the Sample Volume

Signal is normally proportional to the volume, so doubling the volume will approximately double the signal. Stay below 4 mL when using soft beads, and below 10 mL when using hard beads.

Revisit Solid Phase Selection

The solid phase used can have dramatic effects on both signal level and NSB. If you have not already evaluated different solid phases consider trying others. See Tech Note 222 *Solid Phase Selection Guide (TN222)* for more information.

Change the Label

Sometimes changing the label can help increase signal or reduce non specific binding. Tech Note 223 *Labeling Strategies (TN223)* will help in selecting the best labeling method. **Note:** *changing the label can be very productive, however it is seldom productive to change the label volume, flow rate, or concentration.*

If you are labeling through a HIS tag, be aware that Anti-HIS antibodies show significant differences depending on location of the HIS tag on the protein. If one Anti-HIS antibody does not produce good signals, another Anti-HIS may work better.

Reduce the Flow Rate

A slower flow rate increases the contact time and capture percentage of free CBP resulting in larger signals. There are two potential problems with this approach. First, the capture percentage may exceed the maximum recommendation of 30%. See Tech Note 200 *Receptor Valency Effects (TN200)*. Second, the contact time may become long enough to result in a KinExA mode violation. See Tech Note 221 *KinExA Mode (TN221)*. To guard against this first problem we recommend you measure your capture percentage as described in How to Guide 250 *Capture Percentage Test (HG250)*. For accurate measurements the capture percentage at the flow rate you're using should not exceed 30%. Increasing the signal by changing the flow rate is most effective when the initial capture percentage is low.

Kinetic Exclusion Assay requires the contact time with the solid phase be less than the dissociation time of the complex. Reducing the flow rate increases the contact time, thus lowering the maximum off rate to remain in the KinExA mode. With the default flow rate of 0.25 mL/min the off rate will safely be in the KinExA mode when the K_d is tighter than about 10 nM. Even for the slowest flow rate supported (0.025 mL/min) systems with K_d values less than about 1 nM should not have KinExA mode issues. If you have concerns, a KinExA mode test is quick and easy.

Run It With a Small Signal

It may be best to just run experiments with a small signal. Although having a big signal is nice, it is signal to noise that is important as explained in Tech Note 228 *Signal versus Signal to Noise (TN228)*. Reproducible data has often been obtained with Sig100 values of 0.1 volts. Your chance of success here is much better if your NSB level is well below your maximum signal.