Reducing Non Specific Binding

Some systems exhibit high non specific binding (NSB) values. Although NSB is an analysis fit parameter and is subtracted from the signals, noise still contributes to greater uncertainty in the measurements. If NSB is a significant percent of the total signal, it is worth some effort to reduce it before continuing.

Note: Some NSB strategies may interfere with one another so test them individually to avoid skewed results.

Identifying and understanding the source of NSB.

- **Previous Studies:** Did this system show high NSB in other experiments? For example, if an ELISA assay with this system showed high NSB, the KinExA® assay may also show high NSB. Any strategies that worked for ELISA will likely help in the KinExA assay.

- **Solid Phase:** One of the most effective methods to reduce NSB is to change the solid phase. Tech Note 222 *Solid Phase Selection Guide* (**TN222**) shows which solid phase alternatives may be appropriate. The solid phase can have a large change in the specific signal so it is a good idea to look at the results in terms of the net signal (Signal - NSB).

- **Label:** The label you use may effect the NSB values. To see if the label has NSB, run a sample without CBP. If it does, try a different label. There are a variety of different labels available that are subclass specific, heavy and light specific, or cross adsorbed. We've had good results with labels from Jackson Labs (jacksonimmuno.com). See Tech Note 223 *Labeling Strategies* (**TN223**) for more information.

- **Titrant:** If the NSB is coming from the titrant, there is usually a characteristic signature of the signal increasing at the highest titrant concentrations. See Tech Note 210 *Titrant Related NSB* (**TN210**) for an example of this. If the titrant has a HIS-tag, sometimes reversing the assay and using the HIS-tagged molecule as the constant binding partner (CBP) will help. This will reduce the amount of HIS-tag in the samples. Otherwise refer to the Add imidazole section in this document.

Other NSB Strategies

- **Add or increase the amount of blocking protein:** Normally, 1 mg/mL of BSA is recommended but a higher concentration can be tried. Other proteins such as casein or milk sometimes work better. Commercial blocking buffers (SuperBlock, SEA BLOCK, or Protein-Free Blocking Buffer) from Life Technologies (www.lifetechnologies.com) are also available.

- **Add a detergent:** Tween 20 (sigmaaldrich.com) at a concentration of 0.01% can be helpful. Detergent can be used in the samples and/or the label. If the solid phase is adsorption coated it should not be used in the bead vial as it will cause the solid phase coating to desorb. There are many other detergents available. Life Technologies' website has some good information to help select a detergent for a particular system (search for "Detergents for Cell Lysis").

- **Increase the salt concentration:** Most biological buffers contain salt at a concentration of 100-150 mM. In some cases it has been effective to have a higher concentration salt wash after the sample or label. More salt can also be added to the sample buffer. Caution should be used as more salt can interfere with the binding being measured, especially at concentrations over 500 mM. When testing higher salt concentrations, either in the buffer or as a wash, be sure to test the effect on signal as well as NSB.

- **Add heparin:** Heparin has been used at concentrations of 50 to 500 µg/mL. In some systems it may work at even lower concentrations. The pH of the buffer should be checked after adding heparin.

- **Add imidazole:** Imidazole is a functional group on histidine and can be particularly helpful for systems that have a HIS-tag. Sometimes it also helps with non-HIS tag systems that exhibit high NSB. We usually run imidazole at 10 mM concentration. The pH of the buffer should be checked after adding imidazole.