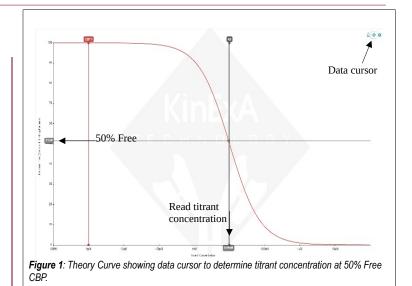# KinExa Mode Test

In a Kinetic Exclusion Assay (KinExA®) the underlying principal is that the contact time of any portion of the sample to the solid phase is shorter than the dissociation rate of the complex. This kinetically excludes competition from occurring between the solid phase and solution phase materials. Being "in the KinExA Mode" means there is not significant competition occurring and the signal is proportional to the free constant binding partner (CBP) in the sample. If the system is out of the KinExA Mode, the solid phase captures not only free CBP but CBP that was complexed with the titrant. Consequently a higher signal, and thus an inaccurate percent free CBP will be reported. Measurements conducted under these conditions will cause the K_d to appear weaker than it actually is. Read TN221 for more information. This guide will explain how to perform a KinExA Mode Test when needed. Consider conducting a KinExA Mode test if preliminary Range Find results indicate a K_d weaker than single digit nanomolar.

Because the signal will change with each flow rate, a Sig 100 and NSB will need to be run to calculate the percent free for each flow rate. For the inhibited point select a titrant concentration that results in nearly 50% free CBP. For each flow rate calculate the percent free for the inhibited point using the following formula:

[Percent free] = ((Signal-NSB)/(Sig100-NSB)) x 100   Equation 1.

Selecting the 50% Inhibited Point

The titrant concentration that yields a 50% Free CBP point can be found using the Theory Curve or from the Range Find experiment. Follow the y-axis down until you reach the 50% free CBP point then follow the point across to the best fit curve. Drop the point from the curve to the titrant concentration on the x-axis. Read the titrant concentration from the x-axis. The Theory Curve has a data cursor in the top right corner. The data cursor [❖] may make it easier to find the titrant concentration along the x-axis (**Figure 1**).

Figure 1: Theory Curve showing data cursor to determine titrant concentration at 50% Free CBP.

Preparing the Experiment

Prepare enough sample to run all of the experiments out of the same sample tubes. When the flow rate doubles the sample volume needed also doubles, for example:

Sample Volume	Flow Rate
500 µL	0.25 mL/min
1000 µL	0.50 mL/min
2000 µL	1.00 mL/min
4000 µL	2.00 mL/min

Choose at least two different flow rates to determine if you are out of KinExA Mode. More points may be needed to determine which flow rate is required to be in KinExA Mode. For example, to run an experiment at 0.25 mL/min and 1.00 mL/min the volume required is 2500 µL + dead volume (DV) = 2700 µL/point.

To ensure the entire sample flows over the flow cell at a constant rate, the flow rate for the Buffer step after the sample draw must be adjusted to match the flow rate of the sample draw (**Figure 2.**) Notice as the **Rate** increases the **Volume** automatically adjusts to the volume required. The **Rate** for the label draw does not need to be changed.

Run each flow rate as its own experiment from the same sample tubes.

Calculate the Percent Free CBP using **Equation 1** for each flow rate. You need to run faster flow rates until the % Free CBP stops changing.

Note: When using soft beads (eg. Azlactone) include 15 seconds of no flow at the beginning of the sample run, and 60 seconds at the end of the last wash step. This allows the soft beads to expand to their normal volume (flow compresses them) before the signal is calculated (Figure 3).

Draw Source		Time (sec)	Volume (uL)	Rate (mL/min)	Titrant Concentration	Time Stamp
Sample Set 101-102	▼	120	2000	1.0000 ▼		⏱
Rack 1: Tube 1		120	2000	1.0000 ▼	0	
Rack 1: Tube 2		120	2000	1.0000 ▼	0	
Buffer	▼	30	500	1.0000 ▼		
Standards: Tube 1	▼	120	500	0.2500 ▼	0	
Buffer	▼	30	125	0.2500 ▼		
Buffer	▼	90	1500	1.0000 ▼		
	▼	0	0	0.0000 ▼		

Figure 2. Sample Timing file shows the Buffer step outlined in red that needs to match the flow rate of the Sample Set.

Draw Source		Time (sec)	Volume (uL)	Rate (mL/min)	Titrant Concentration	Time Stamp
Buffer	▼	15	0	0.0000 ▼		
Sample Set 101-103	▼	120	2000	1.0000 ▼		⏱
Rack 1: Tube 1		120	2000	1.0000 ▼		
Rack 1: Tube 2		120	2000	1.0000 ▼		
Rack 1: Tube 3		120	2000	1.0000 ▼		
Buffer	▼	30	500	1.0000 ▼		
Standards: Tube 1	▼	120	500	0.2500 ▼	0	
Buffer	▼	30	125	0.2500 ▼		
Buffer	▼	90	1500	1.0000 ▼		
Buffer	▼	60	0	0.0000 ▼		
	▼	0	0	0.0000 ▼		

Figure 3: Sample Timing file shows the additional no flow steps required for soft beads when running fast flow rates.