

Capture Percent Test

In a KinExA® assay, only a fraction of the free CBP (Constant Binding Partner) in solution is captured by the solid phase. This How To Guide describes a simple experiment to quantify the percent captured.

The signal level inside a KinExA bead pack decays exponentially with distance, as indicated in **Figure 1**. To estimate the percentage of the CBP captured in the first half of the bead pack, all that is necessary is to measure the signal at two different spatial points in the bead pack. The simplest way to do this is to run a two point (Sig 100 and NSB) Signal Test using a normal bead pack height, then run the same Signal Test with **double** the bead timing. It is important to keep all other factors including experiment samples, Sample Timing, and Flow Rates the same for both experiments. Once run, divide the total signal (Sig100 minus NSB) of the double bead pack by the total signal of the normal bead pack and take the square root. This number is the uncaptured percent, subtracting this from 1 will give the estimated capture percent.

$$\text{Capture Fraction} \approx 1 - \sqrt{\frac{\text{Double Bead Pack Signal}}{\text{Normal Bead Pack Signal}}}$$

For accurate measurements, Sapidyne recommends staying under a capture percentage of 30%. In the unlikely event that the capture percent is too high, the easiest remedy is to run the experiment at a higher Flow Rate; see *Receptor Valency Effects in KinExA Measurements (TN200)*. The double bead pack test can be run at the higher Flow Rate to ensure the capture percent is in the range desired prior to running a full experiment.

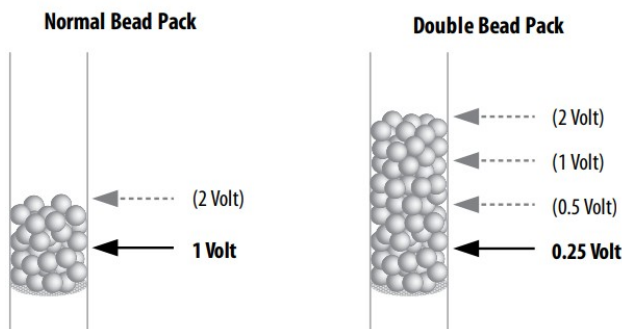


Figure 1. Image of the bead pack in the Flow Cell with a normal bead pack and a double bead pack.

Note: When measuring capture percent for Soft Beads, include 15 seconds of no flow at the beginning of Sample Timing (after Bead Handling) and include 60 seconds of no flow at the end of Sample Timing. See Figure 2. This allows the Soft Beads to expand to their normal size (flow compresses them) before the signal is calculated.

Bead Handling (Soft Beads)				
Draw Source	Time (sec)	Volume (ul)	Rate (mL/min)	Stir
Backflush	20	0	0.0000	<input type="checkbox"/>
Buffer	20	500	1.5000	<input checked="" type="checkbox"/>
Particle Reservoir 1	54	900	1.0000	<input checked="" type="checkbox"/>
Buffer	30	500	1.0000	<input type="checkbox"/>
Waste	2	8	0.2500	<input type="checkbox"/>
Buffer	20	0	0.0000	<input type="checkbox"/>
Buffer	9	150	1.0000	<input type="checkbox"/>
	0	0	0.0000	<input type="checkbox"/>

Sample Timing					
Draw Source	Time (sec)	Volume (ul)	Rate (mL/min)	Titrant Concentration	Time Stamp
Buffer	15	0	0.0000		
Sample Set 101-102	180	500	0.2500		
Rack 1: Tube 1	120	500	0.2500	NSB	
Rack 1: Tube 2	120	500	0.2500	0	
Buffer	30	125	0.2500		
Standards: Tube 1	120	500	0.2500		
Buffer	30	125	0.2500		
Buffer	90	1500	1.0000		
Buffer	60	0	0.0000		
	0	0	0.0000		

Figure 2. Sample Timing for Soft Bead Capture Percent Test.