

Capture Percent Test

In a KinExA® assay only a fraction of the free CBP in solution is captured by the solid phase. This How To Guide describes a simple experiment to quantify the fraction 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 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 double bead pack signal by the normal bead pack signal and take the square root. This number is the uncaptured fraction, subtracting this from 1 will give the estimated capture fraction.

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

In the unlikely event that the capture probability is too high (>30%, see Tech Note 200 *Receptor Valency*), the easiest remedy is to run the experiment at a faster flow rate. The double bead pack test can be run at the faster flow rate to ensure the capture probability is in the range desired prior to running a full experiment.

Note: When measuring capture probability for soft beads include 15 seconds of no flow at the beginning of sample handling and 60 seconds at the end of the last buffer step. This allows the soft beads to expand to their normal volume (flow compresses them) before the signal is calculated.

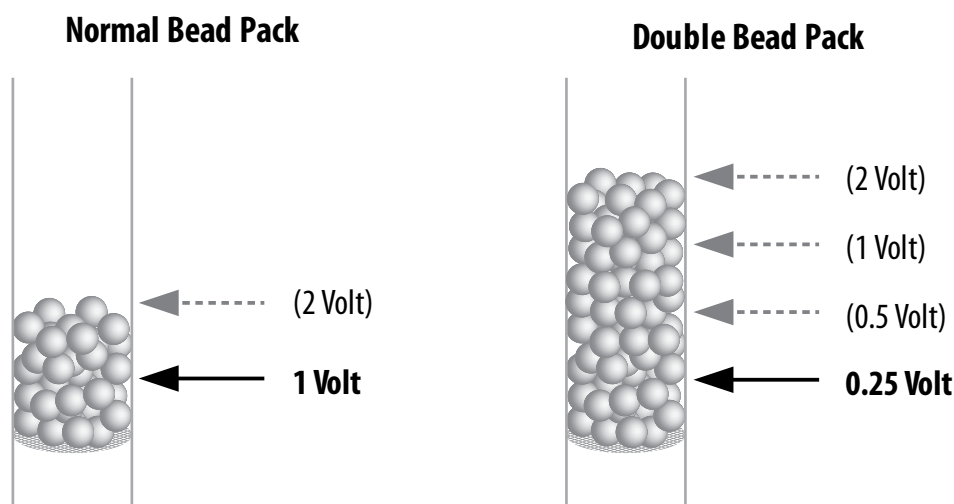


Figure 1. Image of the bead pack in the flow cell with a normal bead pack and a double bead pack.