Sample Preparation for an Equilibrium Experiment

This provides an example for sample preparation for a KinExA® equilibrium experiment. While several methods exist for preparing serial-diluted samples, the following protocol is recommended as it has been found to minimize error.

Step 1: Calculate volumes needed.

Samples

For each sample the volume needed is:

- The volume per run, times the number of cycles **plus** extra sample for charging the lines and dead volume.
  - Charging the lines is a default of 400 µL. This can be eliminated if an Autosampler is used, since no line charging is needed with an Autosampler.
  - Dead volume is usually 100 µL, but it can be reduced to as little as 10 µL with an Autosampler and small volume tubes (see Tech Note **TN206** *Minimum Sample Volumes*).

Label

For the label the volume needed is:

- The volume per run, times number of samples, times number of cycles run, **plus** charging and dead volume.
  - As with the samples, the charge volume can be eliminated and dead volume may be reduced when using the Autosampler.

Sample Buffer

Usually the sample buffer is the same for the samples and label, and is made prior to each experiment. The volume needed is:

- The volume of each sample, times the number of samples **plus** 1 additional sample, **plus** the total volume for the label. The reason for the extra sample is because this is needed for doing the serial dilution in our recommended procedure. Some extra should also be added for pipetting errors and any sample loss in the mixing container.

Step 2: Sample Buffer Preparation

Typically the sample buffer is the same as the running buffer with 1 mg/mL BSA added to reduce nonspecific binding. Sample buffer is used to prepare the experiment samples as well as label solution.

Step 3: Constant Binding Partner Preparation

For an equilibrium experiment, the Titrant is serially diluted in a background of constant binding partner (CBP). Prepare a working CBP solution in sample buffer at the desired concentration and volume. Aliquot the CBP into each sample tube. Make sure to aliquot extra volume in the first tube for the serial dilution.

- e.g. 3 mL in tube 1 and 1.5 mL in all other tubes for a two fold dilution.

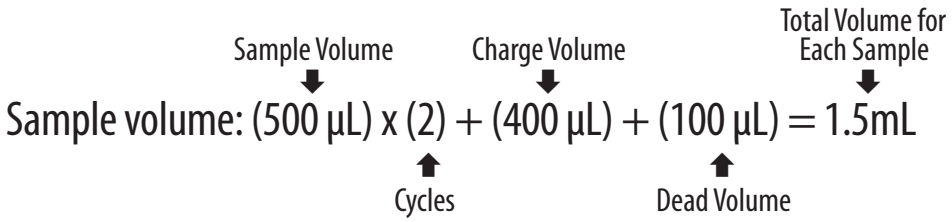
Step 4: Serial Dilutions

Add Titrant to the first tube to achieve the desired starting Titrant concentration. Avoid diluting the CBP significantly. If dilution by more than a few percent cannot be avoided (the Titrant stock is at a low concentration) then the CBP should be supplemented to bring it back up to the correct concentration (see notes).

Mix the tube thoroughly and transfer the appropriate volume to the next tube. Repeat this process for all tubes except the last tube. The last tube should be reserved for CBP only.

Step 5: Equilibration

Allow the samples to equilibrate at the desired temperature (see notes). The amount of time needed to reach equilibrium will depend on the K_d, kinetic constants, and temperature. Once the solutions are at equilibrium, the experiment can be started.



Example (not using an Autosampler). *Using the default timing file, the sample volume is 500 µL and each sample will be run in duplicate.*

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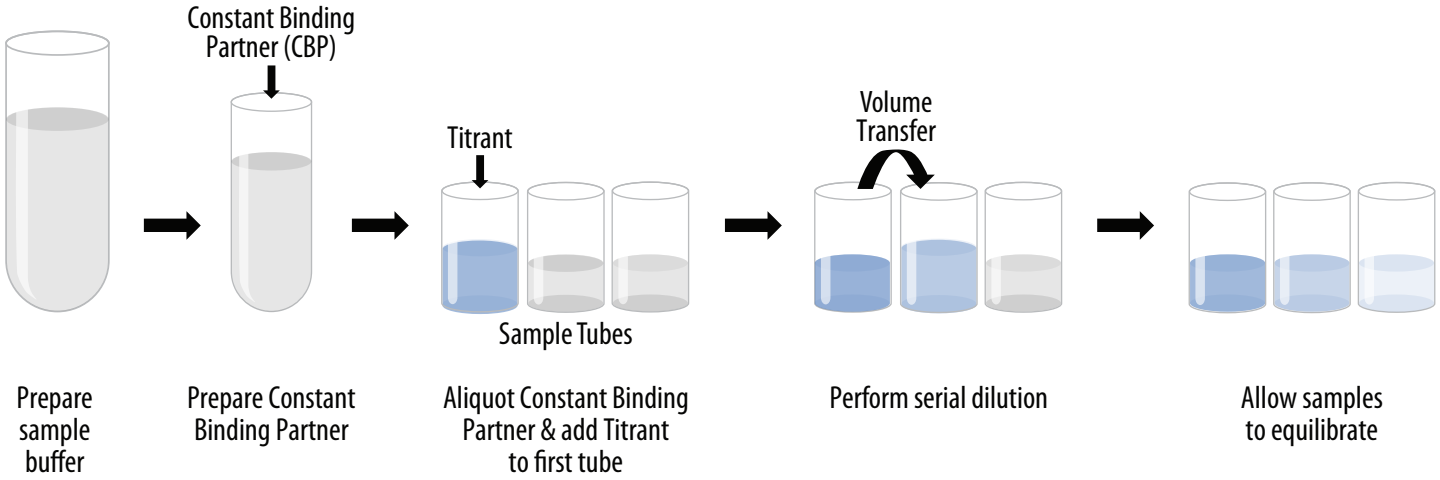

Notes:

CBP Concentration Error

- If the volume of the Titrant added to the first tube is a significant fraction of the total volume of the tube, extra CBP can be added to this tube to bring the CBP concentration back up to the nominal value. For instance, if 100 µL Titrant is added to 2 mL of a 10 pM CBP solution, the final concentration of CBP in solution is now 9.5 pM. To account for the dilution, extra CBP should be added to only the first tube (and not subsequent tubes) to restore the CBP solution to the correct concentration.
- When deciding whether to correct the CBP concentration in the first tube, bear in mind that the concentration error will be diluted (reduced) in the serial dilution process. For the example given, if uncorrected, the CBP concentration in the first tube will be 9.5 pM, the next tube will be 9.75 pM, the third tube 9.875 pM and so on. If these points are in a fully inhibited portion of the curve, they will not cause any error in K_d determination. What is "significant" depends on the experimenter and the goal.

Temperature

- Samples should be run at the same temperature as the equilibration temperature. If the samples are run at a different temperature, the samples will start to shift toward a new equilibrium for the new temperature. It may be necessary to place the entire instrument in an incubator or cold room for certain temperature studies.

Alternative Sample Preparation Techniques

- For large volume samples, please refer to How to Guide **HG231** *Large Volume Serial Dilution* for alternative sample preparation instructions.
- For serial dilutions other than the 2-fold dilution series described, please refer to Tech Note **TN203** *Determining Dilution Series*.