Sample Preparation for Kinetics Injection Experiments

This Tech Note provides sample preparation instructions for a KinExA® Kinetics Injection experiment. For this method the Constant Binding Partner (CBP) and Titrant samples are made separately and then the instrument mixes them together, controlling the reaction time. The following protocol is recommended as it has been found to minimize error:

Step 1: Calculate volume and concentrations needed

CBP

The CBP concentration is made at twice the final running concentration desired, since it will be diluted when injected into the stream of Titrant. The concentration of CBP is recorded in the "before mixing" box as shown in ***Figure 1A***.

The calculation to find the minimum volume of CBP to make is as follows:

Minimum CBP volume = (# of samples) x (Inject volume, *Figure 1B*) x (# of cycles) + (400 µL charge step) + (200 µL dead volume)

Note: *Dead volume accounts for errors made in pipetting and sample loss in the mixing container.*

Titrant Samples

The Titrant concentration is made at twice the concentration desired because it will be mixed with the CBP during the experiment. This is why there is a column for the Titrant concentration before mixing (***Figure 1C***) and a column that shows the concentration after being mixed with CBP (***Figure 1D***). The Titrant will be serially diluted in a background of sample buffer.

For each run, sample is drawn in two sample sets. The first sample set fills the line to the injection T (***Figure 1E***), and the second set mixes Titrant into the CBP sample (***Figure 1F***). These volumes need to be added together to get the total Titrant used per run:

Total Titrant volume per run = (Titrant precharge, *Figure 1E*) + (Titrant volume, *Figure 1F*)

The calculation for the minimum volume per sample is as follows:

Minimum Titrant volume per sample = (Total titrant) x (# of cycles) + (400 µL charge) + (200 µL dead volume)

Note: *Add extra volume to tube 1 for the titration series.*

Label

Since the CBP is placed on the Injection line, the label must be placed on one of the sample lines. It defaults to line 13, as shown in ***Figure 1G***. A different sample line may be used if desired. The calculation for the minimum label volume is as follows:

Minimum label volume = (Label volume) x (# of samples) x (# of cycles) + (400 µL charge) + (200 µL dead volume)

Sample Buffer

The sample buffer used is usually the same for the samples and label and is prepared fresh for each experiment. The minimum buffer volume needed is:

Minimum buffer volume = (Minimum Titrant volume per sample) x (# of samples) + (Minimum CBP volume) + (Minimum label volume) + (Dead volume)

Step 2: Sample Buffer Preparation

The buffer used for sample preparation is usually the same as the running buffer with the addition of 1 mg/mL BSA. This is used to help reduce Non Specific Binding as well as prevent material from sticking to the walls of the container. See Tech Note 219 *Reducing Non Specific Binding* (**TN219**) for more information.

Step 3: Temperature Stasis

Allow time for both the samples and instrument to reach thermal equilibrium. The Kinetic parameters are sensitive to temperature, so steps should be taken to control it. Once the samples are at thermal equilibrium, the experiment can be started.

As an example, a 5 mL sample of buffer at room temperature takes approximately one and a half hours to reach 4°C.

Sample Preparation for Kinetics Injection Experiments

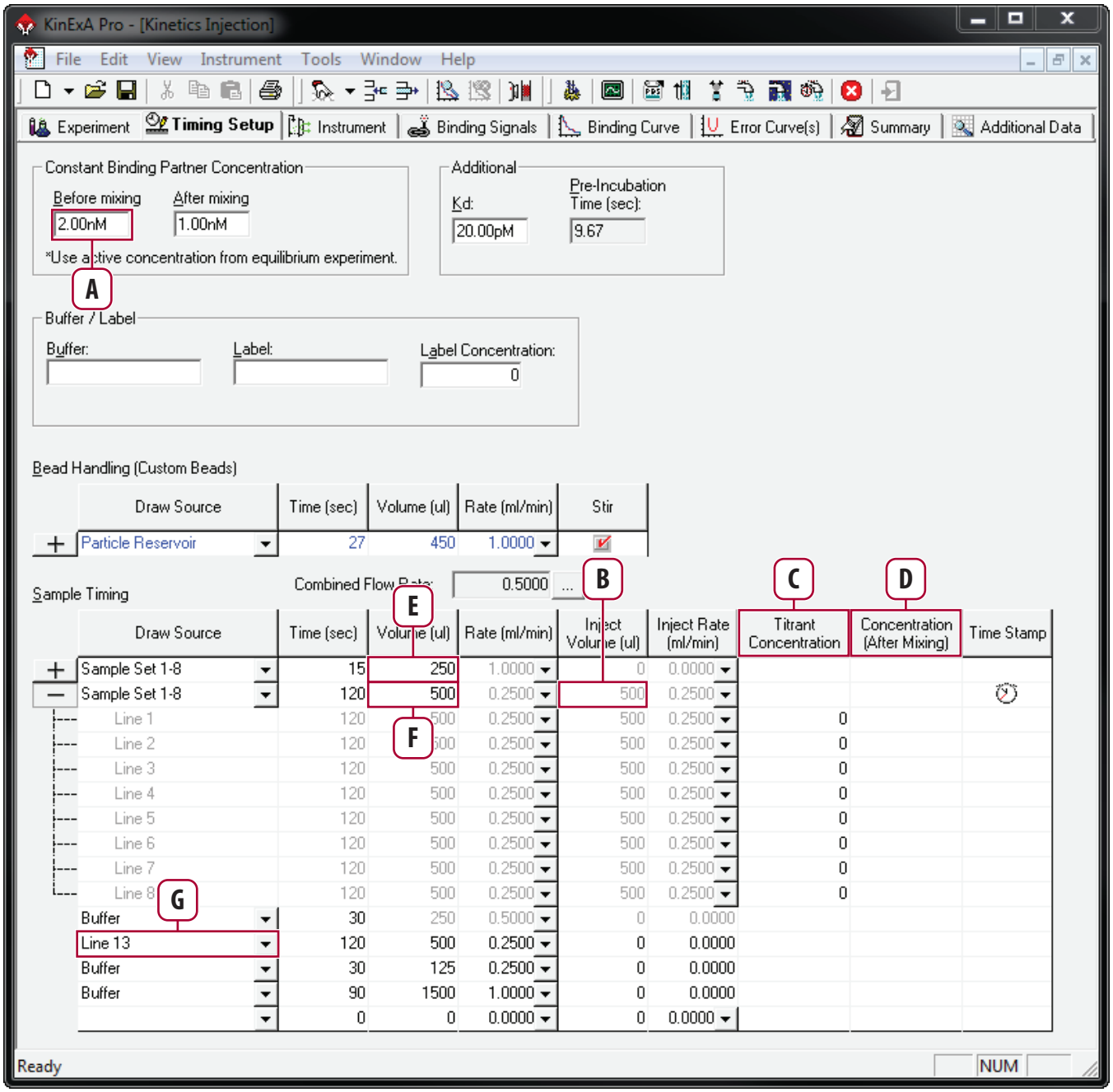

Figure 1. *Parts of the injection sample timing.*