Unequal mixing experiments

Even though the KinTek RQF was designed to do 1:1 mixing, it is possible to do unequal mixing through the use of different drive syringes. For example, by using a 0.5 ml syringe on one side and a 5 ml syringe on the other, one can achieve approximately a 1:10 dilution. However, after the drive syringes have been changed the user must first determine the volume per revolution of the new system.

Example: 1:10 mixing through the use of a 0.5ml syringe. Rather than the typical 846µl/rev of the stock system the volume per revolution will now be roughly 465µl/rev. For all equations used to calculate speed and reaction time the actual measured volume per revolution (measured with option 5 of the scrolling menu) should use the 465µl/rev number.

Calculation of the Reaction Time

The delay line volumes and ranges for reaction times are given in Table 1. These are only approximate figures and will change depending upon your own calibration of this system. The reaction time is calculated simply as the volume of each delay line divided by the speed at which the solution is flowing in µl/ms.

$$t = V/F \quad (\mu l)/(\mu l/sec)$$

$$\text{Reaction Time} = \text{Loop Volume} * 60/(\text{Vol. per Rev} * \text{Run Speed})$$

Sample Calculation (Loop 3):

$$t = (50.9\ \mu l)*60\ sec/min\ /\ (465\ \mu l/rev * 200\ rev/min) = 32.8\ msec$$

Because we use 200 rpm as the minimum run speed, this calculation shows that 32.8 msec is the longest reaction time that can be obtained using Loop 3. To get a 15 msec time point using loop 3:

$$\text{Run Speed} = (50.9\ \mu l)*60\ sec/min\ /\ (465\ \mu l/rev * 0.015\ sec) = 438\ rpm$$

When operating in the delay mode to determine the reaction time, calculate the reaction time as the sum of the delay time plus the time that it would normally take for the solutions to flow uninterrupted through the reaction loop.

Table I. Reaction Loop Volumes

LOOP NO.	LENGTH (cm)	VOLUME (µl)	MAX. TIME (msec)
1	0	16.1	10.4
2	3	35.2	22.7
3	8	50.9	32.8
4	15	85.2	55.0
5	23	133.6	86.2
6	31	169.6	109.4
7	40	199.3	128.6

The approximate volume of each reaction loop is listed. These numbers will vary depending upon the calibration of the instrument as described under *Calibration of the Reaction Loops*. For each reaction loop volume, the number of steps required to expel the reactants and the maximum reaction time obtained at a motor speed of 200 rpm are calculated. Different volumes of quench solution are delivered into the sample collection tube when different reaction loops are used; it may be desirable to add the appropriate volume of quench solution to some of the vials to keep the volume of quench solution constant.

Operation

In order to achieve a 1:10 mixing ratio the larger volume sample must be loaded into the drive syringe because the amount used will be 10x greater than the volume of the sample load loop. Flushing of the lower system will be done using the standard method and once flushed and dry you will load the samples just like a standard experiment using the lower syringe ports and filling solution up to the Minstac connector on the 8 way valve.

Option 3 must be used for unequal mixing runs. Once the motor speed and reaction loop has been determined using the equation above you must tell the system what distance to push. To calculate the distance you must add the step distance of the reaction loop you will be using and add the distance required to expel the mixed sample For the 1:10 ratio in this example the calculation would be as follows, assuming a sample load line volume of 15 µl:

$$\text{Steps} = (4096 \text{ steps/rev} / 465\ \mu\text{l/rev}) * 165\ \mu\text{l} = 1453 \text{ steps}$$

Now you must add this number to the measured step count of the reaction loop that you will use for each reaction time. Enter this number at the second prompt of the speed and distance routine.