KinTek Corporation

Chemical-Quench-Flow
Servo-Motor
Model RQF-3

Instruction Manual

Check our Web Site at

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for up-to-date information and parts list
and online ordering of replacement parts.

7604 Sandia Loop, Suite C, Austin, TX 78735
PH: [814] 387- 4678
www.kintekcorp.com

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I. INSTALLATION OF THE INSTRUMENT

1. There is one cable connection from the instrument to the computer terminal and a power cable that is compatible with 120 VAC or 220 VAC.

2. Calibrate the instrument prior to performing an experiment as described in section VI. The calibration parameters listed in Table I have been entered into the computer at the factory. These are approximate values to enable you to begin testing the operation of the instrument but should not be used for an actual experiment.

SAFETY WARNINGS

1. The servo motor is very powerful. Keep hands and hair away from the motor while it is running.

2. Unplug the unit before removing any access panels or attempting to service the electronics.

3. Use good laboratory safety practices.

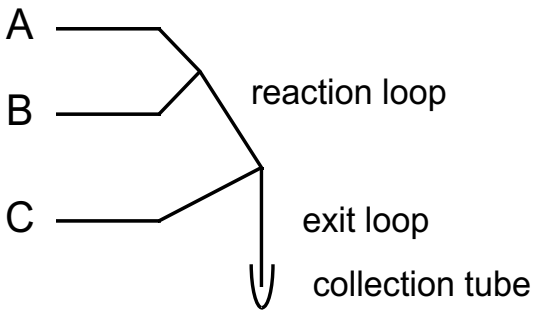
II. PRINCIPLES OF OPERATION

In a 3 syringe chemical quench-flow experiment, a reaction is initiated by the mixing of two reactants. The reaction then continues as the mixed reactants flow through a reaction loop and then are mixed with a quench solution to stop the reaction. The time of the reaction, t, is thus determined by the volume of the reaction loop, V, and the rate of flow, F:

$$t_{(msec)} = V_{(\mu l)}/F_{(\mu l/msec)}$$

To vary the reaction time, you can vary the volume of the reaction loop or the rate of flow. There are practical limits to the range of either of these parameters. The KinTek Quench-Flow is supplied with 7 reaction loops varying from approximately 16 μl to 200 μl, providing reaction times from approximately 2-60 msec in continuous mixing.

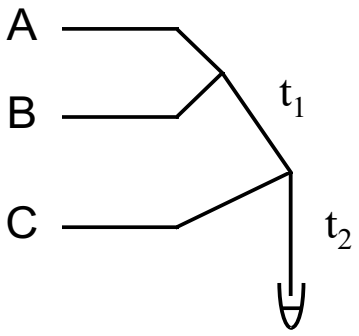
Reactants A and B are first mixed and the reaction proceeds while they flow through the variable length reaction loop. The reaction is stopped by mixing with a quench solution contained in syringe C. The mixture is then expelled into a collection tube.



To achieve reaction times greater than 60 msec, a push-pause-push mode is used; the first push mixes the reactants and pushes them into the reaction loop. The apparatus then pauses for a defined period to allow the reaction to proceed, then a second push expels the reactants, mixing them with the quench solution in the exit line to stop the reaction.

After the system is calibrated (see Section VI), the selection of the proper reaction loop and the setting of proper motor speeds and delay times are under computer control. Simply input the desired reaction time and the computer will display which loop to use and automatically set the appropriate flow rate.

A second delay time can be obtained by holding the reactants in the exit loop prior to expelling them into the collection tube, as shown at the right. The final quenching of the reaction is achieved by mixing with a quench solution contained in the collection tube. To setup a second reaction time, t_2, simply press the "Second Delay" control and the second delay field will appear (see Section III). The computer will calculate the minimum reaction time based on the maximum flow rate and enter this value into the field. If a time that exceeds the maximum time for continuous mixing is entered, the motor will use the push-pause-push method as it does for longer t_1 times.



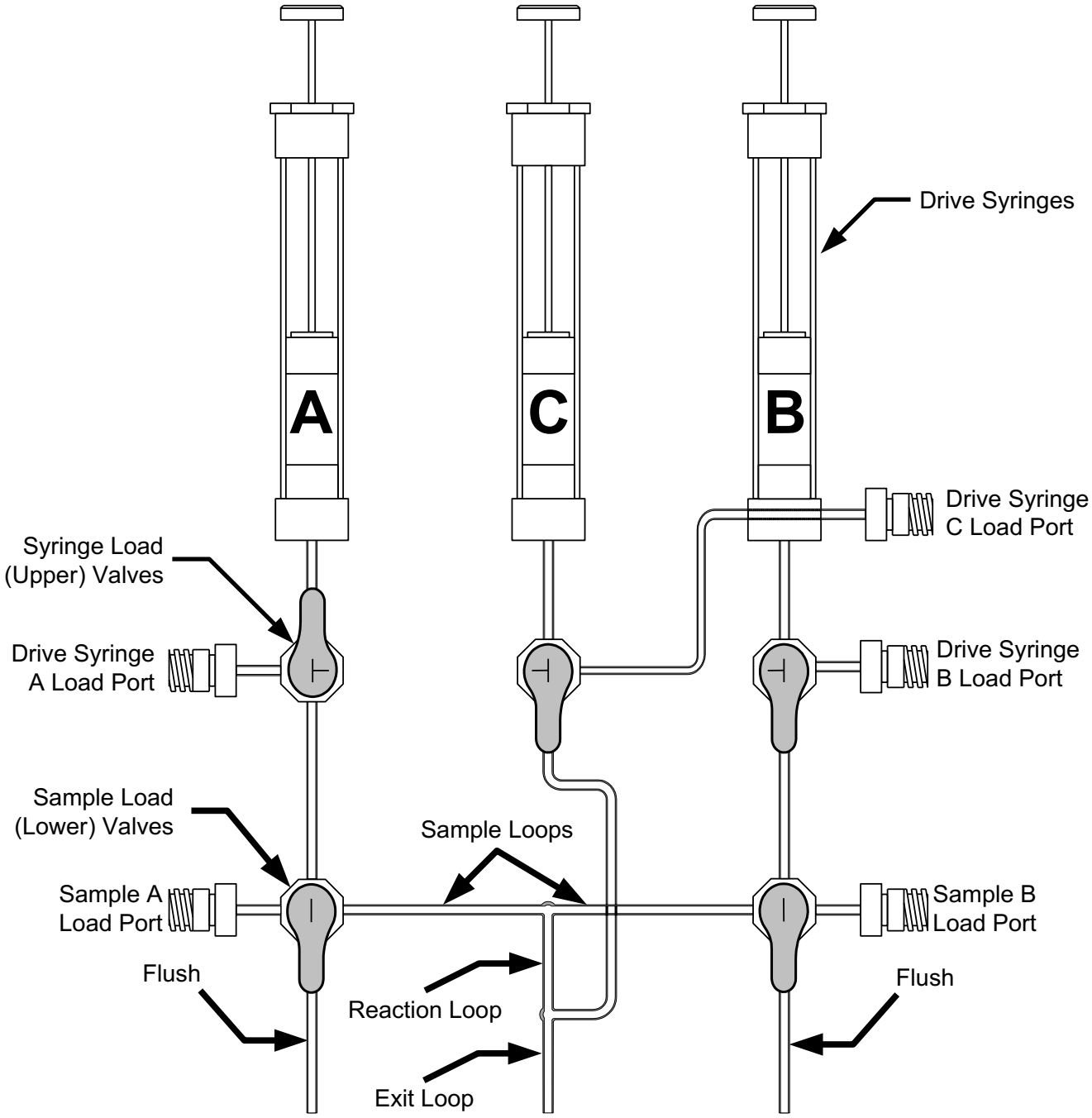


Figure 1a. ***Schematic of the 3 Syringe Quench-Flow Valve System.*** **The valves are shown in the proper position to FIRE a shot.**

III. COMPUTER CONTROL OF THE MOTOR

The KinTek chemical quench flow apparatus allows precisely defined speeds of mixing over programmable distances using a servo motor. The servo motor is controlled digitally producing 8192 steps per revolution.

You are cautioned that this is a very powerful motor! Keep hands and hair clear of the motor and drive shaft while the motor is running!

NOTE: To prevent the drive plate from going beyond the extremes of travel, there are safety limit switches on the drive mechanism. If you engage one of the limit switches, the motor control will inhibit further motion in that direction. The motor will only move in the opposite direction until the limit switch is cleared.

Before operating the motor, it is important that you first become familiar with the operation of the valves used to load samples and flush the lines between runs as described under Section IV. However, before loading the syringes, you may first want to experiment with the computer control of the motor with the drive syringes empty. Movement of the motor is entirely under computer control through the terminal.

Specific instructions for each option of the computer program are presented below. Each screen with the exception of the main screen has an “escape” (ESC) control. The ESC control will display the main screen in all cases except during a sequence (Quench flow run or Large volume quench). Pressing ESC during a sequence will simply abort the sequence.

The main screen of the KinTek Quench Flow terminal is shown below. The specific functions of the controls and how they pertain to the program are outlined in the instructions for each screen of the computer program.

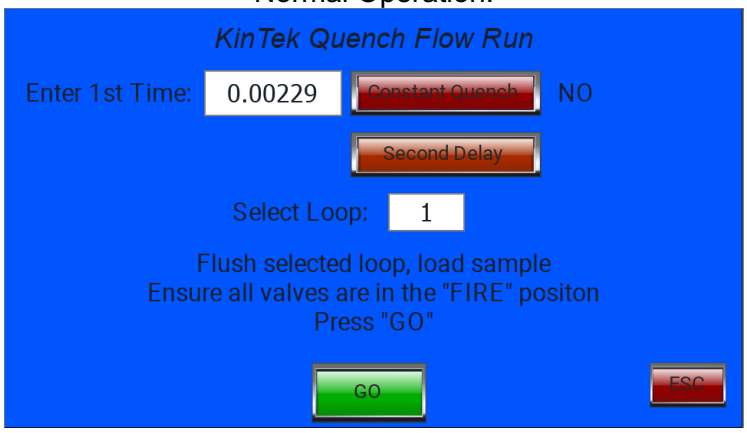
When the system is first turned on it will load and boot up the KinTek RQF control program. The syringe platform will then find the upper limit switch and move back down one revolution. This position becomes “home”.

The KinTek Quench Flow terminal.

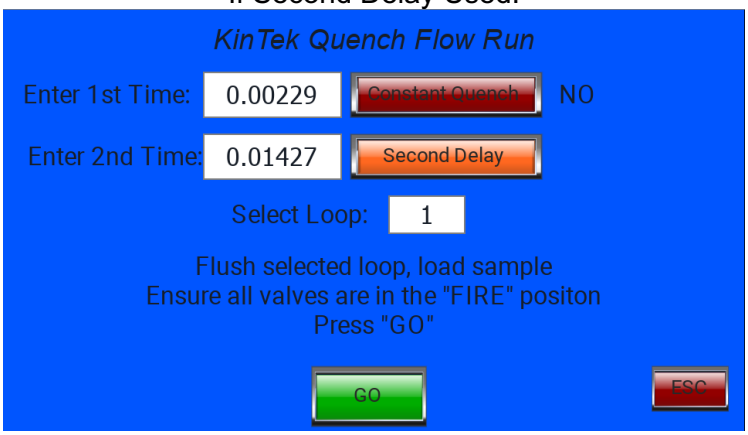
A. QUENCH-FLOW RUN

After calibration parameters have been entered (See “D. Enter/Change Parameters”), this option will be used to perform a normal quench-flow experiment. Depending on the options chosen for constant quench and second delay, one of the following screens will appear:

Normal Operation:



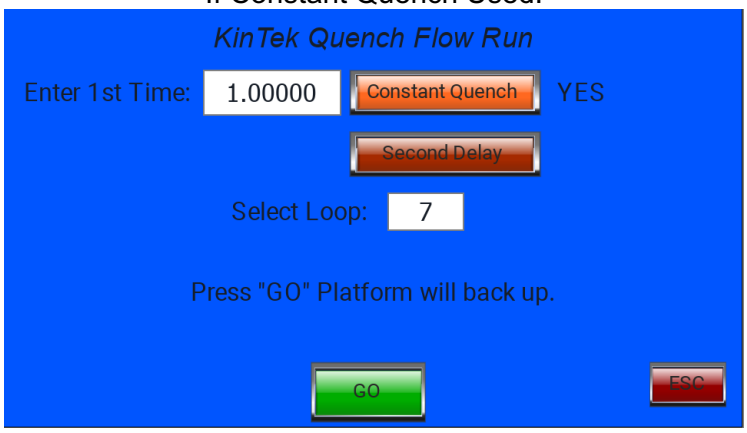
If Second Delay Used:



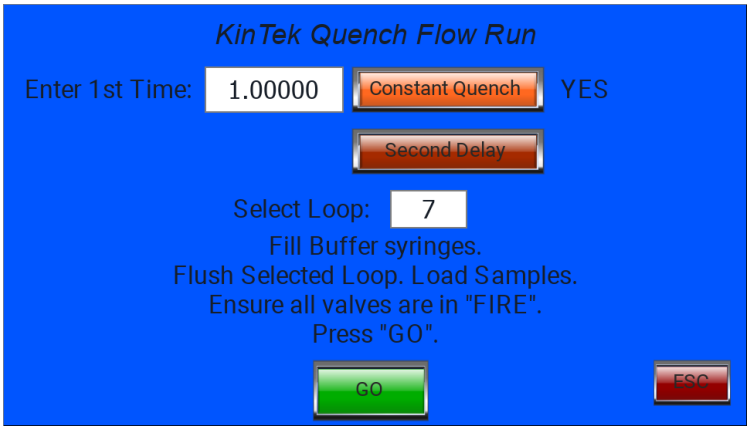
If a time has not yet been entered, the terminal computes the minimum reaction time and enters that value in the field automatically. The minimum values shown depend on your calibration values (See “D. Enter/Change Parameters”) and the maximum speed of the motor. If longer times are desired, touch the field and enter the desired value using the pop-up keypad.

Once the desired value is entered for the 1st time point, the terminal will display the required loop for the entered value based on the desired time and a range of flow rates.

If Constant Quench Used:



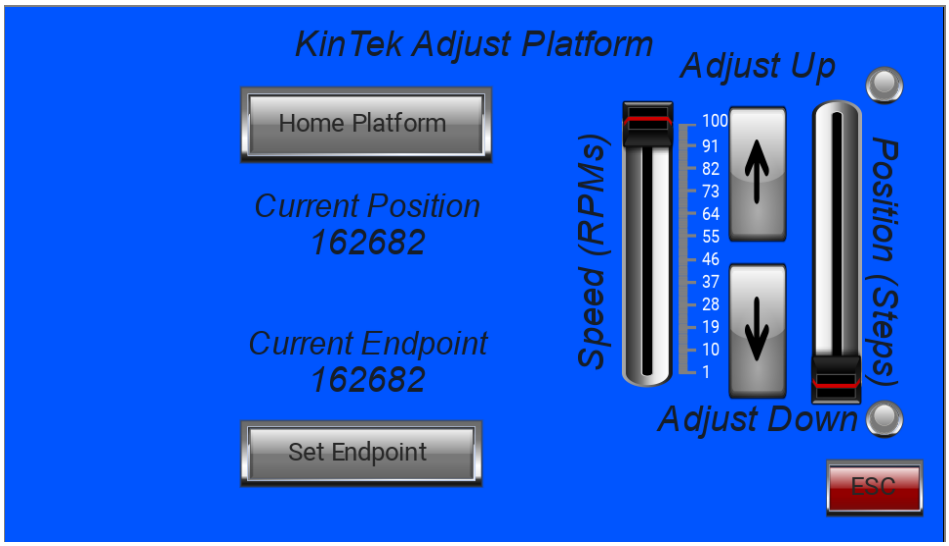
If constant quench is used and the selected loop is not loop 1, the program will prompt for GO to move the platform. When GO is pressed, the program will back up the required amount to ensure all loops use the minimum quench volume required for loop 1 and will then prompt to fill the buffer syringes, flush the selected loop, load samples, ensure all valves are in “FIRE” and press GO again.



Press the GO key on the keypad to start the reaction. Press ESC to abort the run after it has started and return to the Quench Flow Run Setup screen. Pressing ESC while on the Quench Flow Run Setup screen returns the terminal to the main menu.

B. ADJUST PLATFORM

Use this screen to adjust the position of the motor drive plate up and down.



The “Home Platform” control will move the platform to the home position automatically.

The “Set Endpoint” control will set a new endpoint based on the current position of the platform.

The “Speed” slider determines the speed of movement when adjusting the platform. Speeds from 1 to 100 revolutions per minute (RPMs) are available.

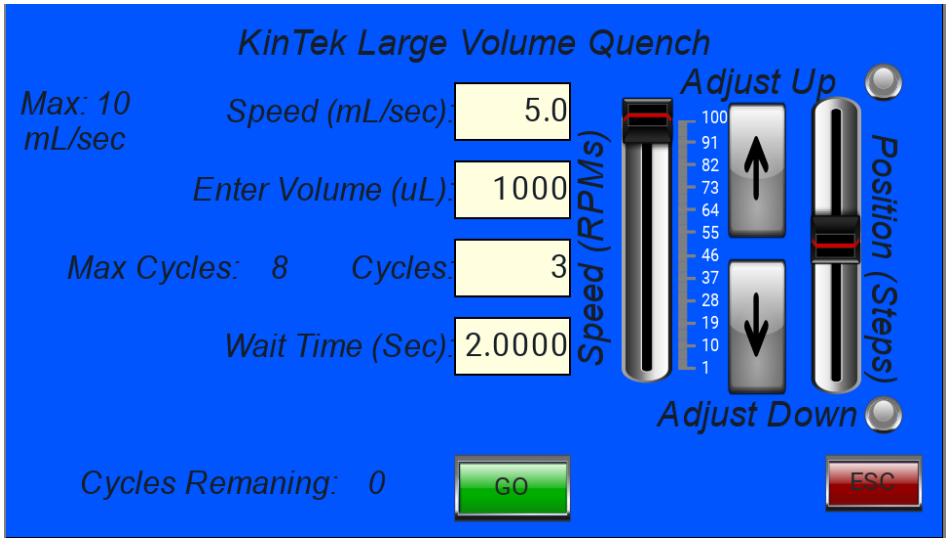
There are up and down arrows for continuous platform adjustment and a position slider that can be used. The slider position on the scale reflects the current position of the platform. Moving the slider will move the platform to the same relative position once the slider has been released.

There are also 2 indicators on the top and bottom of the position slider that will “light” (change color) when the platform has reached a maximum travel and engages the upper or lower limit switch. Engaging the upper or lower limit switch will inhibit further movement of the platform in that direction. Adjust the platform in the opposite direction to disengage the switch.

Pressing ESC from the adjust platform screen will return the terminal to the main menu.

C. LARGE VOLUME QUENCH

This option is used to gain direct control over the speed, volume, and number of repeat pushes of the platform.



Speed (mL/sec) – Enter the desired motor speed in mL/sec (milliliters per second). The maximum flow rate is 10 mL/sec. A typical speed for the quench flow run is 6 mL/sec. The terminal will calculate the appropriate motor speed for a given flow rate (See section VII. QUENCHING LARGE VOLUMES).

Volume (µl) – Enter the number of micro-liters to push. The program will calculate the exact distance to push based on the volume per revolution variable. This variable is entered in the parameters screen and is calculated when calibrating VPR (See section F. CALIBRATE SYRINGES). The pop-up keypad will display a maximum volume based on the current platform position.

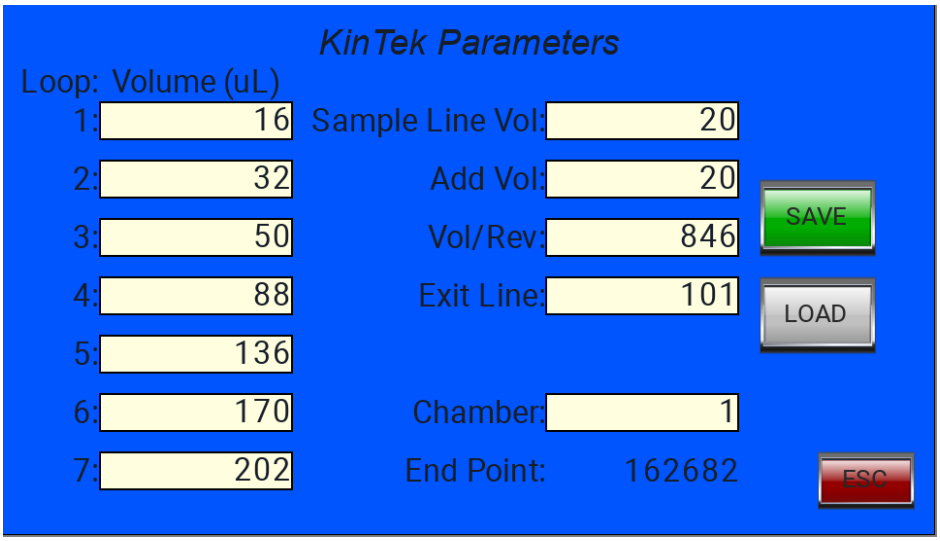
Cycles – Enter the number of times you want a push-pause-push cycle to be repeated. If "1" is entered here, the terminal will not require a wait time. It will simply push the desired volume. A maximum number of cycles will be displayed based on the volume entered and the current platform position.

Wait Time (Sec) – Enter the time delay between push cycles, in seconds. A countdown of cycles remaining will display as the reactions are pushed through the system.

Adjustment controls are also available on this screen for convenience (See section B. ADJUST PLATFORM").

Once these parameters have been entered, press GO to start the reaction(s). Press ESC to abort the run and return to the Large Volume Quench Setup screen. Press ESC on the Large Volume Quench Setup screen to return to the main menu.

D. ENTER/CHANGE PARAMETERS



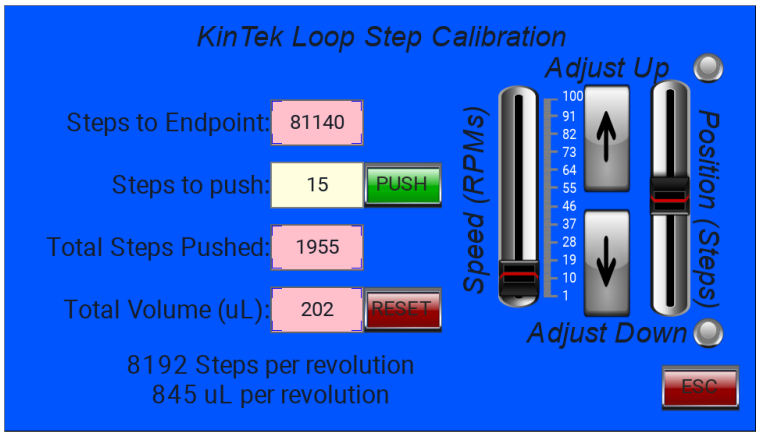
This option is used to enter the calibration parameters. These parameters, volume (μl) for each reaction delay loop, the sample volume (μl), the volume delivered per revolution of the motor (μl) and the exit line volume (μl) are used to determine the speed and distance to push during quench flow runs. Default parameters are entered at the factory, but it is HIGHLY recommended that calibration volumes for a specific syringe chamber are entered before performing a quench-flow experiment. Once these calibration parameters have been entered, the user must press the “SAVE” button or they will revert to their previously saved values. Once saved they are stored in the hand-held terminal. It is not necessary to modify them or re-enter them unless a change has been made to the syringe chamber that affects the system calibration.

Multiple syringe chambers can be saved in the static memory of a single terminal. The current, loaded syringe chamber is displayed upon opening the screen. Changing the chamber number will enable the “LOAD” button if calibration parameters exist for the entered chamber. If they do not exist within the system, enter the calibration values for a given chamber and press “SAVE”.

Syringe chamber calibration is covered in Section VI of this manual. The user can also fine tune the amount of solution collected by using the “Add Vol” parameter to have the system push a desired additional volume.

Press ESC to exit to the main menu.

E. LOOP STEP CALIBRATION



This option can be used to confirm your calibration values or as an alternate method of loop volume calibration. Load the two drive syringes with buffer and leave the quench syringe (middle syringe) empty and turned off. Then flush and dry the SAMPLE LINES and REACTION LOOPS as described in Sections IV-V. Be sure to place the valves in the FIRE position after flushing. This calibration is based upon determining the number of steps required to expel the air from the SAMPLE LINES and REACTION LOOPS to bring the buffer just up to the tip of the EXIT LINE. This can be done in increments and the program will sum the total distance moved and the total volume pushed which is calculated using the following formula:

D(VPR/SPR) = Volume Pushed

Where D = Measured Distance in steps
VPR = Volume per Revolution
(See F. CALIBRATE SYRINGES)
SPR = Steps per Revolution = 8192

Steps to Endpoint: Displays the number of steps available until the endpoint is reached. This is based on the current platform position.

Steps to push: Enter the desired number of steps to push each time the “PUSH” button is pressed.

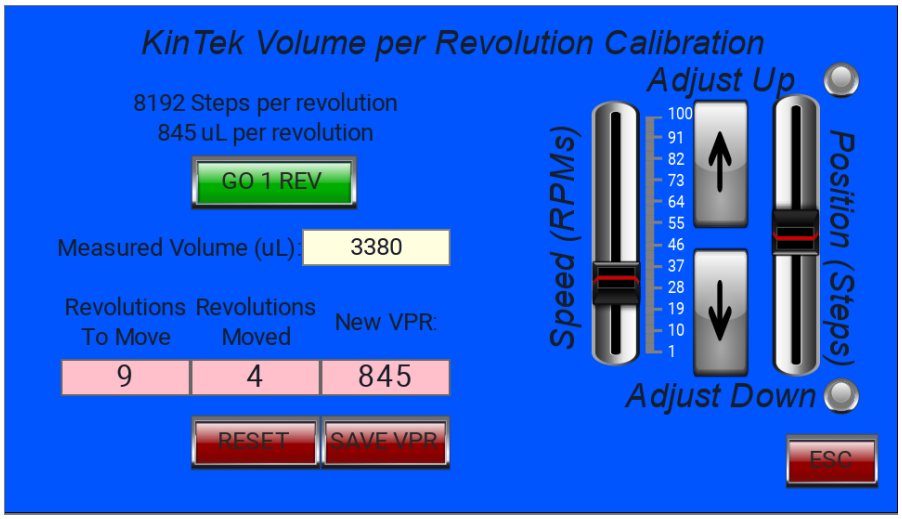
Total Steps Pushed: The cumulative number of steps pushed since the screen was loaded or since the “RESET” button was pressed.

This screen also displays the steps per revolution and the current calibrated volume per revolution in microliters.

Platform adjust controls are also available on this screen for convenience (See “B. ADJUST PLATFORM”).

Press ESC to exit to the main menu.

F. CALIBRATE SYRINGES



This option allows a simple calibration of the volume delivered from the two drive syringes for one revolution of the motor. This volume should be approximately 845 µl. First, load the two buffer drive syringes with water or buffer, removing all air bubbles. DO NOT load solution into the quench syringe (the middle syringe). Place the valves in the FIRE position and advance the drive plate to expel solution from the exit line until all bubbles are removed (do this using the adjustment controls provided). Press "GO 1 REV" to advance the platform 1 revolution and collect the delivered solution from the exit line into a weighed vial. It is recommended to push several revolutions for a more accurate measurement. After each push, the display will show the number of revolutions moved and the revolutions to move based on the current platform position. Once several pushes have been collected in the vial, weigh it again to get a precise volume measurement. Enter this volume into the "Measured Volume" field. Once the measured volume is entered, the system calculates the new VPR automatically and displays it in the "New VPR" field. Press "SAVE VPR" to save the new VPR in the system. It will also automatically be entered in the calibration screen (see "D. ENTER/CHANGE PARAMETERS"). Press "RESET" to reset the count of revolutions back to zero if redundant measurements are desired.

Press ESC to exit to the main menu.

G. HOME SYRINGE DRIVE

This control is used to move the drive plate to a preset position near the upper limit of the syringes. When the instrument is powered on, the plate will back up until it touches the upper limit switch and then it will advance one revolution to the HOME position. Subsequently, the motor will go directly to the HOME position when this control is pressed.

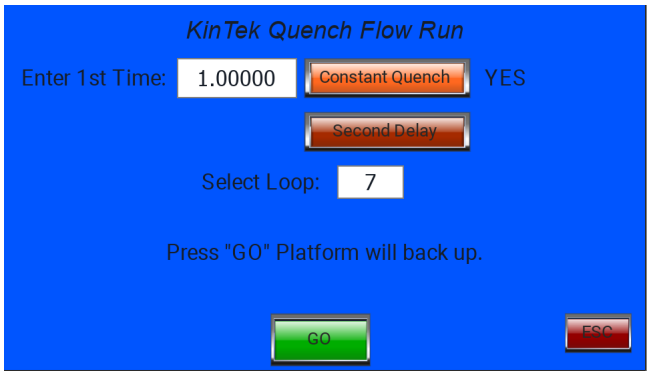
H. CONST QUENCH, SECOND DELAY

i. Constant Quench

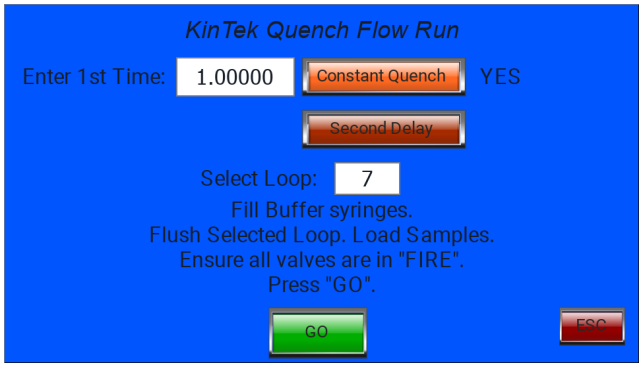
Normally the quench flow will deliver a larger volume of quench solution for the longer delay lines because the quench syringe is pushed while the reaction loop is being filled. In order to obtain a constant quench volume, the syringe drive platform will back up before each run after you enter the reaction time if any loop other than loop 1 is selected. The quench volume delivered from the quench syringe will then be the same for each reaction loop. This is the minimum quench solution possible, equal to the volume normally delivered through loop 1.

Unless the selected loop is number 1,
You must manually fill the buffer drive syringes to bring their plungers up to meet the drive platform.

During a quench flow run using constant quench, the terminal will prompt to press “GO” to back up the platform:



then prompt again for the buffer syringes to be filled before firing if the selected loop is not loop 1:

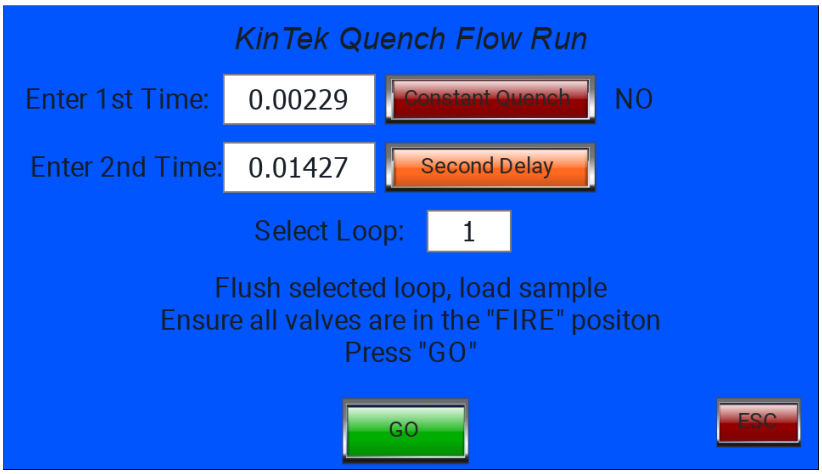


With each shot, the system will continue to prompt for the platform to back up, then buffer syringes to be filled and fired when “Constant Quench” mode is enabled.

ii. 2nd Quench Delay

After the reactants have been quenched by mixing with the solutions from the third syringe, they can be held in the exit loop for a defined period of time prior to expulsion into the collection tube where the reaction can be stopped by mixing with a quench solution (i.e., acid or base) in the collection tube. For example, this is useful in doing pulse-chase experiments where you may quench with an excess of unlabeled substrate, allow sufficient time for 5-6 turnovers of the enzyme, then expel the reactants into a solution containing acid or base to terminate the reaction.

On the Quench Flow Run screen, press the “Second Delay” control and the terminal will display a field to enter the desired second delay time.



IV. VALVE ARRANGEMENT

Figure 2 shows the schematic arrangement of the valves of the Three Syringe Quench Flow. Each drive syringe is loaded via a 3 way valve. In addition, each sample can be loaded via a separate sample valve. Although you can load reactants into the drive syringes as in a conventional quench-flow apparatus, this leads to considerable waste of valuable reagents. The KinTek Quench-Flow was designed to allow small sample volumes ~15 μL to be loaded and recovered with nearly 100 % efficiency as described below.

Examine the valves on the front of the instrument and you will note that on each of the small valve handles there are lines to indicate the arrangement of the holes through the valves which make connection to various plumbing lines. The three valves across the top are used to load the drive syringes with buffer (A & B) or quench solution (syringe C) whereas the sample valves at the bottom are used to load the samples and flush between runs.

The two valves at the bottom differ from the upper ones. In the lower sample valves, the outflow goes through the back of the valve and the single line points to the plumbing connected to the outflow.

Figure 2 shows the valves in various positions during operation. To load the drive syringes, place all three syringe valves in the horizontal position (Fig. 2a). In the FLUSH position (Fig. 2b), suction can be applied to the exit line and used to draw buffer, methanol and then air up through the valves to purge the sample loops, reaction loops and the exit line. To load reactants into the sample loops attach a syringe onto the lower side ports and turn the line on the sample valve to point toward the syringe (SAMPLE LOAD position, Fig. 2c). After placing the valves in the FIRE position (Fig. 2d), solution from the drive syringe forces the reactants out of the sample loops and through the selected reaction loop in the 8-way valve and out the exit line. The 8-way valve is used to select one of 8 reaction loops (only 1 shown for simplicity) to vary the reaction time as summarized in Table I in section VI.

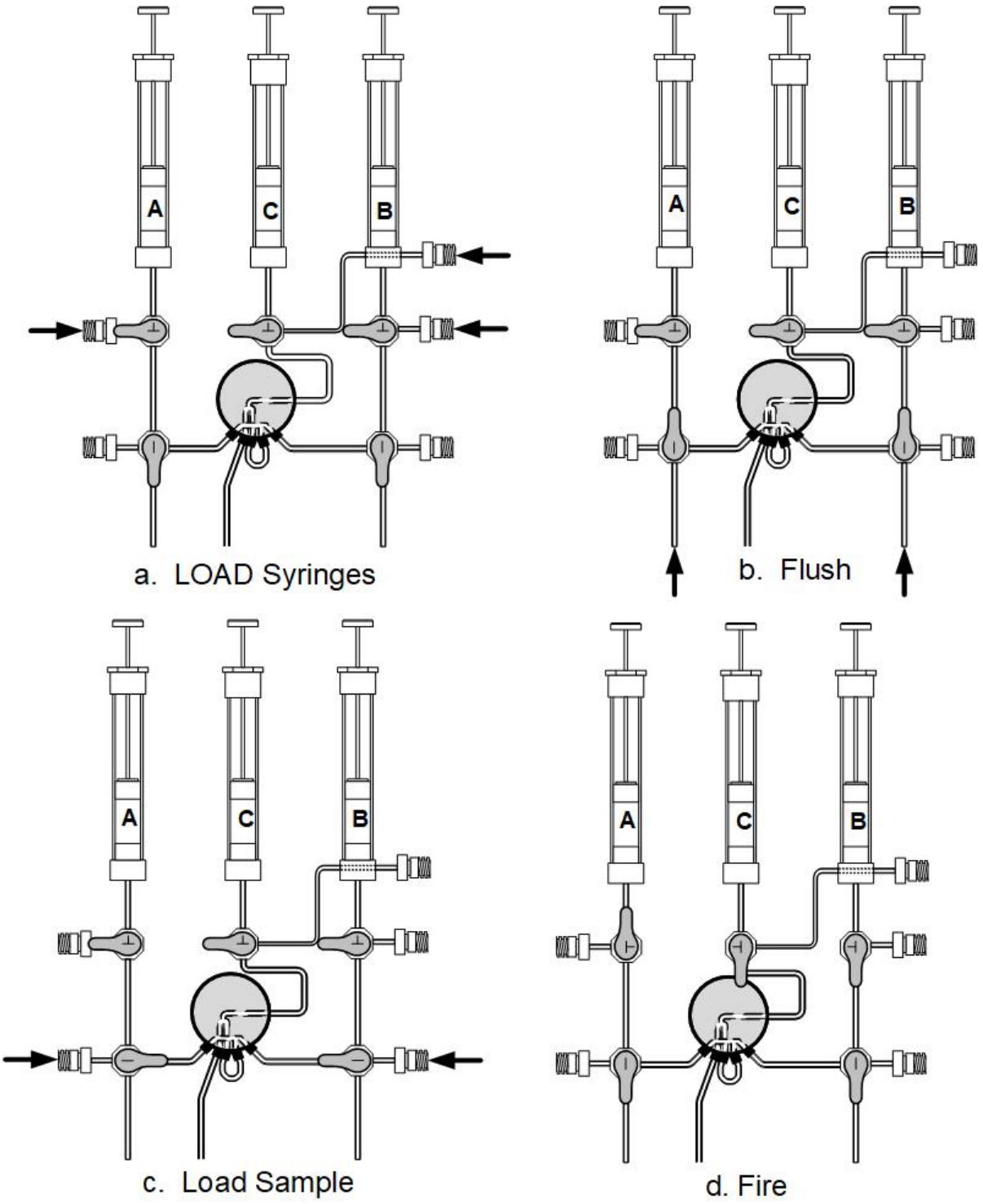


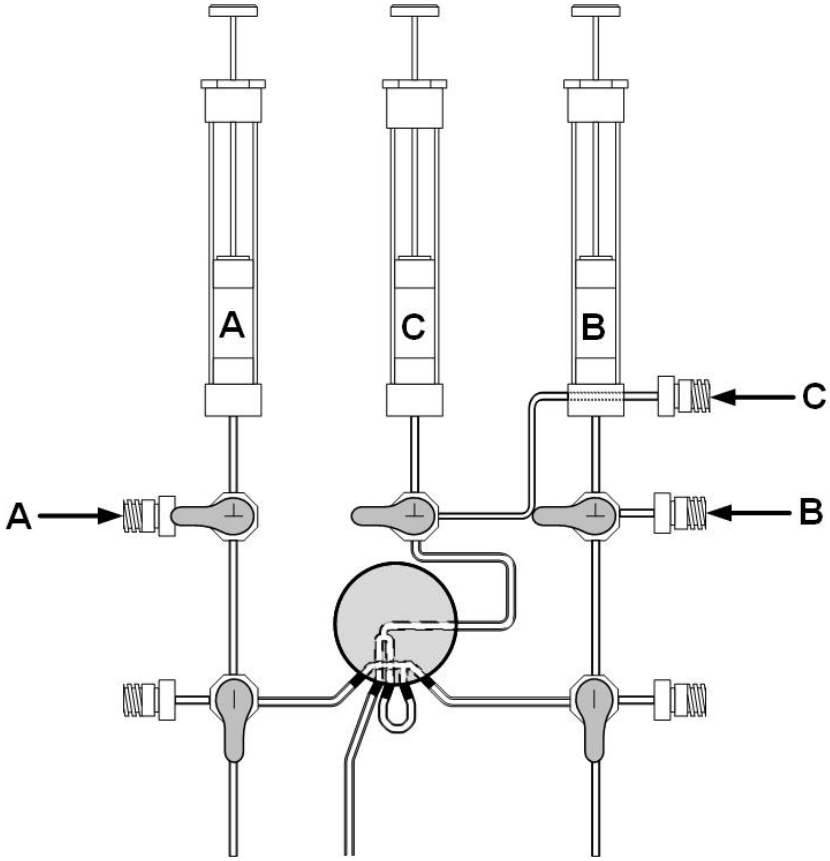
Figure 2 ***Valve Positions for Quench-Flow Operation.*** **The valve positions are shown for loading, firing and flushing sample lines. The small arrows indicate the ports used for loading or flushing.**

V. SAMPLE LOADING PROCEDURES

A. Load the Drive Syringes

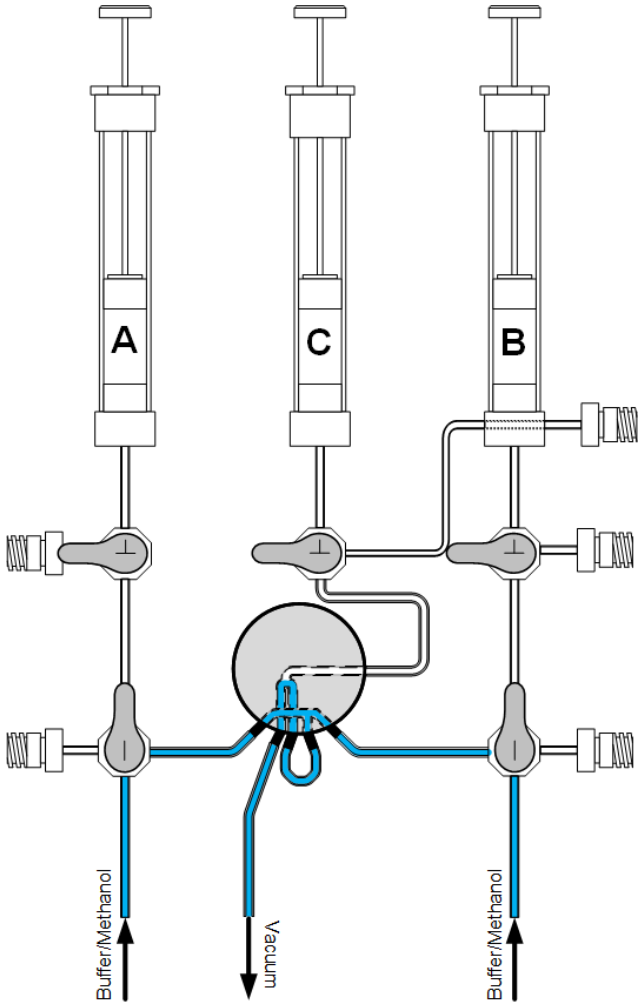
In order to begin an experiment, you first load the drive syringes with buffer (syringes A & B) and quench (syringe C) solutions. Do this by turning all three of the upper valves to their horizontal position which allows connection to be made only between the side ports for attaching syringes and the drive syringes. Load all three of the drive syringes while keeping the loading valves all in the horizontal position until you are done. This will prevent solution, especially the quench solution, from backing up into any of the other syringes.

To remove air bubbles from the drive syringes while loading with buffer and quench solution, the best method is to work the solution back and forth rapidly, pausing to allow air bubbles to rise in the external syringe and then again forcing solution rapidly up into the drive syringe and back out again. This can very effectively and efficiently remove all of the air bubbles from the drive syringe. Now turn the valves to the FIRE position and bring the drive plate down to contact the drive syringes and force a small amount of buffer out of the exit line.



B. Flush the Sample Lines

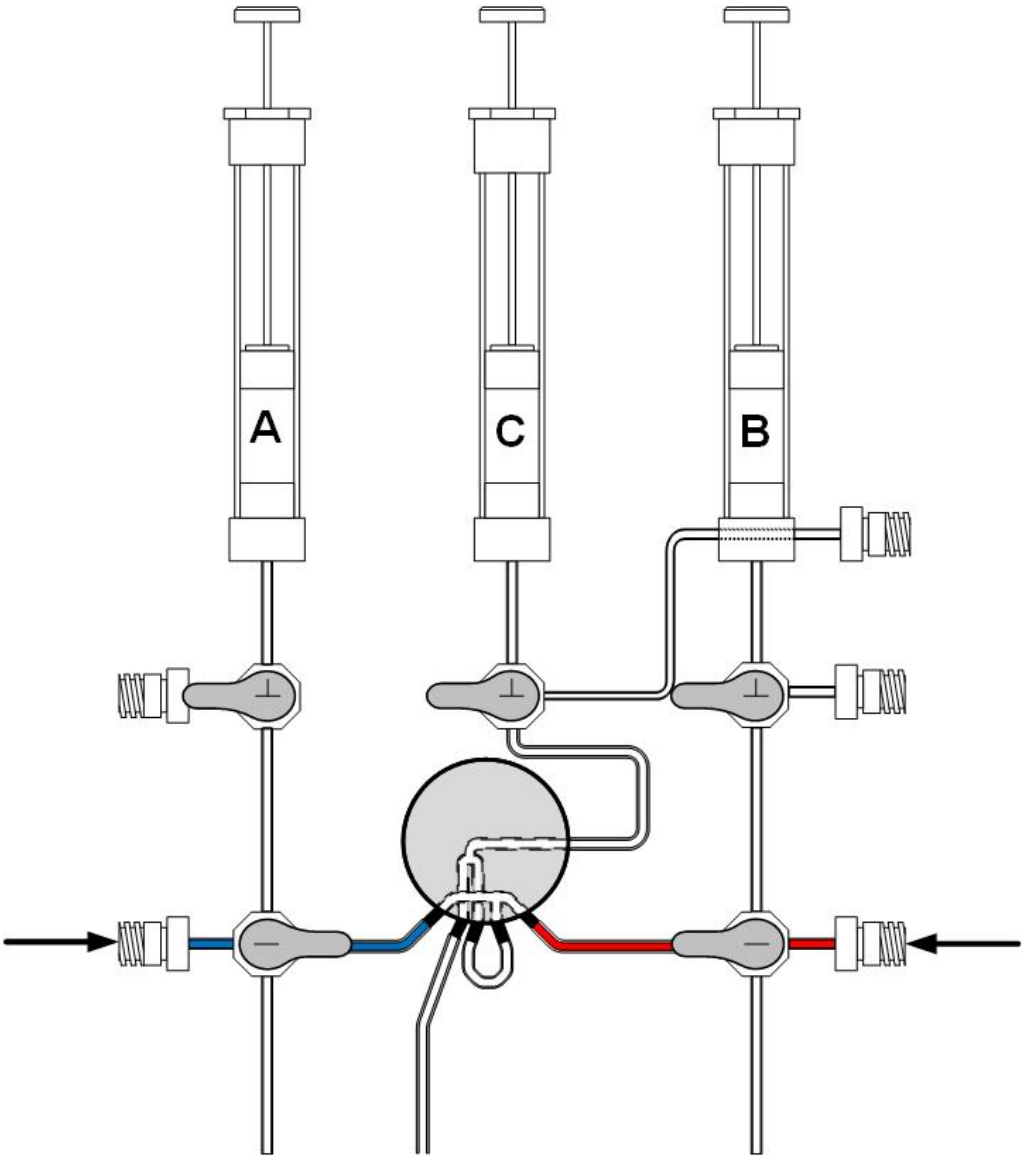
After loading the drive syringes, flush the lower half of the plumbing. Flushing is done by turning the lower sample load valves to the FLUSH position and then applying suction to the exit line. The two flush lines then can be immersed in water and then methanol while applying a suction to draw solution up through the valves, through the loading lines and the reaction loop and out the exit loop. Following the methanol flush, continue sucking with air until the lines are dry (20-60 sec). Depending upon the nature of the quench solution and the temperature, you may need to turn the center syringe valve to the horizontal position to prevent quench solution from being sucked into the exit line. Remember to change the valve back to the fire position after flushing. Prior to loading your first samples, you should also flush the load lines. Turn the lower valves to the LOAD position and, while applying the suction, use a pipette to dribble buffer and then methanol into the lower syringe ports on the side of the apparatus. Continue sucking air until the syringe ports are dry.



Valves shown in the “FLUSH” position.

C. The Load-Fire-Flush Cycle

To begin an experiment attach a 1 ml syringe containing your samples to the lower load ports.



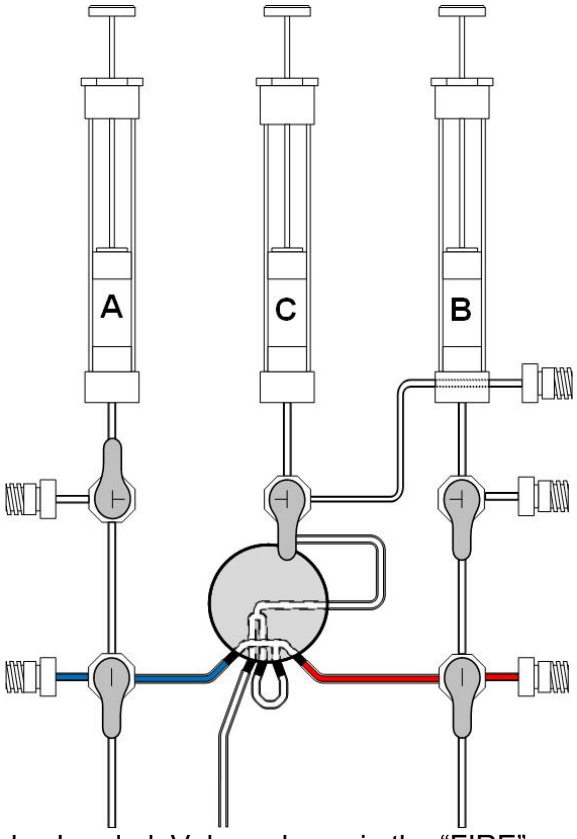
Load samples through the lower load ports. Valves shown in the “LOAD” position.

For each time point, the following sequence is used.

1. Turn the valve to point toward the sample syringe (load position) and load the samples one at a time, bringing the meniscus of the sample just up to the tubing connection on the eight-way valve. After loading one sample, turn that valve to the fire position so that the valve is connected to the drive syringe line. Load the second sample in the same manner and move that valve into the fire position as well.

2. Hold a tube to collect the sample so that the tube coming from the exit line is at an angle against the side of the tube. This helps prevent splashing of the sample out of the tube. We have also found it useful to punch a small hole in the lid of a 1.5 ml Eppendorf tube and insert the exit line through the hole to collect the sample. This is most easily accomplished with a nail heated in a Bunsen burner (in a fume hood!).

3. Make a final check to see that all valves are in the proper position to FIRE. Firing with the valves in the wrong position will force solution through the closed valve, causing damage to the valve.



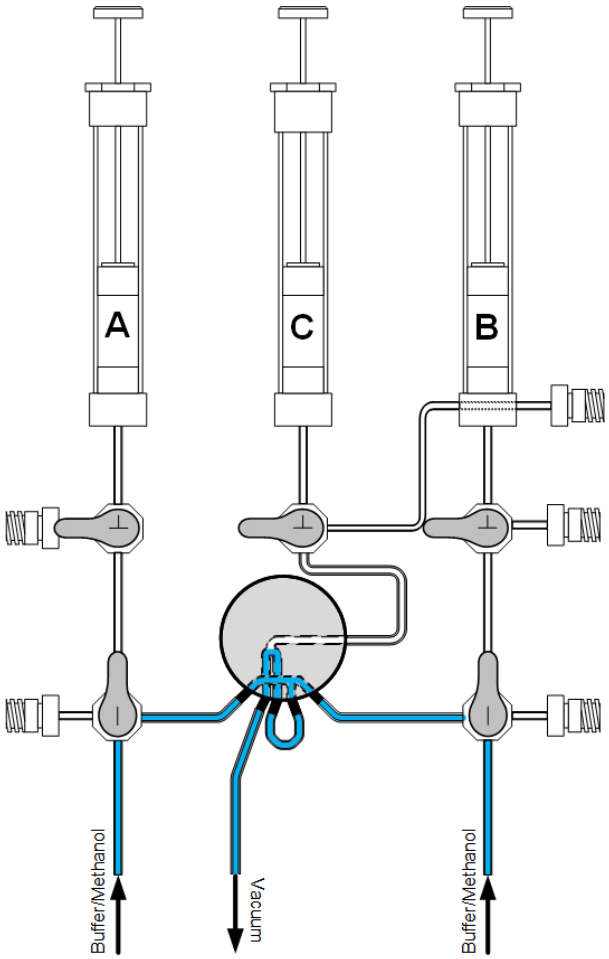
Samples Loaded, Valves shown in the “FIRE” position.

4. Push GO on the screen to start the reaction (see Section III). The servo motor will drive the syringes to force the reactants together through the reaction loop, exit line and into the collection tube.

5. Flush the lines by the following sequence:

i. Attach the vacuum line to the exit line from the quench flow but do not yet turn on the vacuum.

ii. Turn each of the sample valves to the FLUSH position and then turn on the vacuum pump. Immerse the flush lines first into buffer, then methanol and then allow 20-60 seconds to air dry.



iii. Disconnect the vacuum line. You are now ready to load the next sample. If you need to change the position of the 8-way valve to select a new reaction loop, do so now and repeat the flushing with buffer, methanol and air.

This cycle can be completed within about 2 minutes so that an entire time course consisting of 25 data points can be obtained from 1 ml of solution can be done within about 45 minutes.

VI. CALIBRATION OF THE REACTION LOOPS

A. Volumes of Reaction Loops and Sample Lines

The principal of operation for measuring the volume of each loop is shown in Figure-3. The volumes of the reaction loops and the sample lines need to be calibrated precisely. This is done by loading a solution of radioactive or absorbance standard into each loop and then flushing to recover the solution contained within. By counting or measuring the volume and absorbance of the sample, you can precisely measure the volume of that loop. The volumes to be measured are between 15 -300 μl.

1. Calibration method using radioactive standard

This is best done using ^{32}P-phosphate because you can collect samples in water and count directly by the Cerenkov method without the need for scintillation fluid. Make up a solution of ^{32}P-phosphate in phosphate buffer giving 10,000 - 20,000 cpm when 200 μl is diluted into 10 ml of water and counted on the tritium channel. Using this solution, the volumes of each of the loops can easily be determined with less than 1% error.

i. Using a lower sample load port, load one of the sample lines with radioactive solution such that you fill the sample line, reaction loop, and the exit line entirely and excess solution comes out of the exit line. You have now primed one side of the system with radioactive sample as shown in Figure-3a.

ii. By pushing buffer from the QUENCH loading port, you will force out the contents of the exit line. Counting that sample will provide a measurement of the volume contained within the exit line. You are then left with radioactive sample distributed as shown in Figure-3b.

iii. By flushing with a solution from the other sample line or from the opposite BUFFER loading port, you can expel the contents of just the reaction loop. This sample provides a measurement of the volume contained within that reaction loop. Now radioactive sample is contained only in the sample line originally chosen for loading as shown in Figure-3c.

iv. By flushing with buffer from the BUFFER loading port on the same side, you will then expel the contents of that sample loop and thereby determine its volume.

By repeating this process, you can determine the volumes of the two sample lines, each of the eight reaction loops, and the exit line. By doing each in triplicate, you can determine these volumes with high precision (1% error). This provides you with the numbers necessary to determine the reaction times given by each of the reaction loops.

Compute the volume of each internal loop according to the formula:

$$Vol = cpm_{\text{sample}} \cdot \frac{200\ \mu L}{cpm_{\text{standard}}}$$

2. Calibration method using an absorbance standard

Calibration by use of an absorbance standard requires accurate measurement of both the absorbance and volume of solutions recovered from the quench-flow instrument following a load/flush cycle described below. Start by measuring and recording the weight of 5 ml test tubes. Prepare an absorbance standard so that when the 200 µl of standard solution is diluted into 5 ml, it gives an absorbance of 0.5. Weigh the test tube before and after adding the stock solution and the water to get an accurate measurement of the total volume. Perform the dilution and measurement of the standard in triplicate.

i. Load one of the sample lines with the absorbance standard solution so that you fill the sample line, the reaction loop, and the exit line entirely and flush excess solution out of the exit line; flushing with about 2 ml of solution is usually adequate. You now have the absorbance standard sample completely loading the lines as shown in Figure 3a.

ii. By pushing buffer from the QUENCH loading port, you will force out the contents of the exit line. Flush with 2-5 ml, weigh the sample to determine the volume recovered and measure the absorbance. This sample will provide a measurement of the volume contained within the exit line. You are left then with absorbance standard distributed as shown in Figure 3b.

iii. By flushing with a solution from the other sample line (one not used to load the absorbance standard sample) or from the opposite BUFFER loading port, you expel the contents of the reaction loop. Measure the volume and absorbance of this sample. This will provide a measurement of the volume contained within that reaction loop. Now absorbance solution is contained only in the sample loop originally chosen for loading (Figure 3c).

iv. By flushing with buffer from the same BUFFER loading port, you will expel the contents of that sample loop and thereby determine its volume.

By repeating this process, you can determine the volumes of the two sample lines, each of the eight reaction loops, and the exit line. By doing each in triplicate, you can determine these volumes with high precision (1% error). This provides you with the numbers necessary to determine the reaction times given by each of the reaction loops.

Compute the volume of each internal loop according to the formula:

$$Vol = A_{\text{sample}} \cdot Vol_{\text{sample}} \cdot \frac{200\ \mu L}{A_{\text{standard}} \cdot Vol_{\text{standard}}}$$

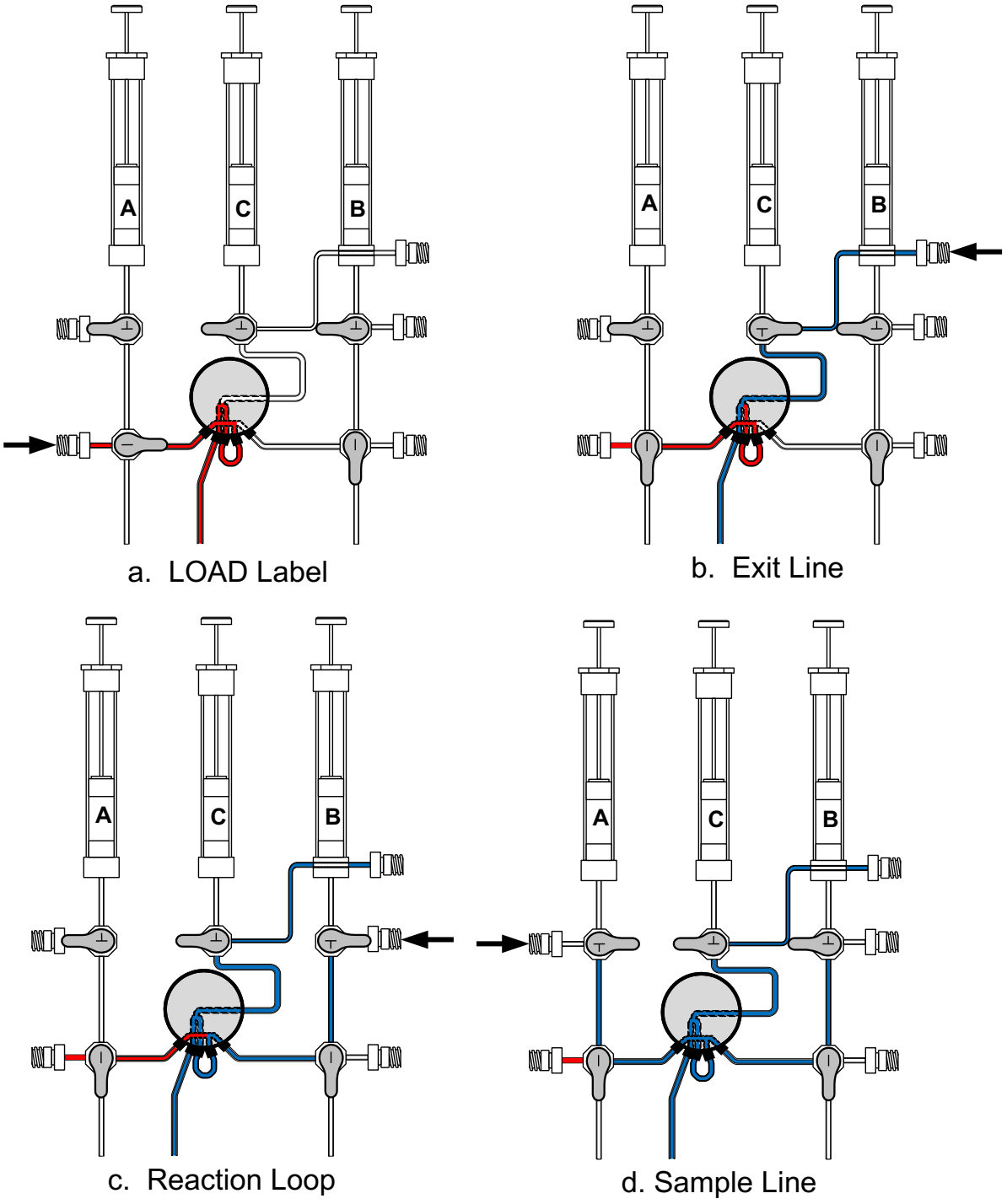


Figure 3. ***Calibration of Sample and Reaction Loops.*** **The method for calibrating the sample loops and reaction loops is outlined in this scheme. The sections of tubing containing radioactive sample or absorbance standard are shown in red. The small arrows show the ports used to first load and then flush the various tubing segments. Pay close attention to the valve positioning in each figure.**

B. Sample Line Volumes

To determine the volume delivered by each of the sample lines, load each sample line with the ^{32}P-phosphate standard or absorbance standard solution as if you were performing an experiment, bringing the meniscus up to the metal connector on the sample loop as described under Sample Loading Procedures. Then move the lower valves to "FIRE" and flush with buffer from the upper buffer syringe loading port. Collect the contents into a scintillation vial or into a test tube and count or weigh and measure the absorbance if an absorbance standard is used. This will yield the precise volume loaded into the sample lines each time an experiment is performed. Note that the Teflon tubing has a capacity of approximately 5 μl per centimeter of length, so an error of +/- 1 mm in loading the sample would generate an error of 0.5 μl in the sample load volume.

C. Syringe Volume Delivered per Revolution

The final step of the calibration is to establish the volume delivered per revolution of the motor. This is done by loading 2 of the Buffer drive syringes and priming the fluid system, then driving the syringes multiple motor revolutions (4 to 5 are recommended), and collecting the buffer or water in a test tube which has been pre-weighed. This is accomplished using the Volume Per Revolution screen of the program (see Section III). By weighing the test tube before and after expelling the solution into it, you can accurately determine the volume delivered. Using the 5 ml syringes, this should give about 846 μl per revolution for 2 syringes.

D. Calculation of the Reaction Time

The reaction loop 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 reaction loop divided by the speed at which the solution is flowing in μl/msec.

$$t_{(msec)} = V_{(\mu l)}/F_{(\mu l/msec)}$$

The calculation is done by the computer automatically according to the following equation:

Reaction Time = Loop Volume * 60/(Vol. per Rev *Run Speed)

Sample Calculation (Loop 3):

$$t_{(msec)} = (51_{(\mu l)} * 60_{(sec/min)}) / (846_{(\mu l/rev)} * 220_{(rev/min)}) * (1000_{(msec)} / 1_{(sec)}) = 16.4_{(msec)}$$

Because we use 220 rpm as the minimum run speed for quench flow runs, this calculation shows that 16.4 msec is the longest reaction time that can be obtained using Loop 3. To get a 15 msec time point using loop 3:

$$\text{Run Speed} = (50.9_{(\mu l)} * 60_{(sec/min)}) / (846_{(\mu l/rev)} * 0.015_{(sec)}) = 240 \text{ rpm}$$

When operating in the delay mode to lengthen the reaction time, the computer calculates 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)	STEPS	MAX. TIME (msec)
1	0	16	1704	5.2
2	3	35	1888	11.3
3	8	51	2043	16.4
4	15	85	2372	27.4
5	23	134	2846	43.2
6	31	170	3195	54.8
7	40	200	3485	64.5
8	not used			

The approximate volume of each reaction loop is listed. These numbers will vary dependent upon the calibration of the instrument as described in section VI., A. Volumes of Reaction Loops and Sample Lines. For each reaction loop volume, the number of steps required to expel the reactants and the maximum reaction time obtained at a minimum motor speed of 220 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.

VII. QUENCHING LARGE VOLUMES

Although the instrument was designed to minimize sample volumes, it can be used in a more conventional quench-flow mode by loading samples directly into the drive syringes. This is more convenient for quenching larger, preparative scale sample volumes. For example, suppose you wish to mix 2 ml of enzyme with 2 ml of substrate, allow the reaction to proceed for 10 msec to form an intermediate, and then quench to stop reaction. This reaction can be run in one push of the quench flow using the Large Volume Quench screen as outlined below.

First, calculate the flow rate in mL/Second to obtain a 10 msec reaction time. For quenching large volumes, you should maintain a flow rate in excess of 2.5 mL/second or 180 RPM of the motor so a sample loop should be selected to obtain a sufficient speed. This limit is calculated based on the default 846 μl/revolution of the motor and a minimum recommended speed of 180 RPMs as follows:

$$\text{MinFlowRate}_{(mL/sec)} = ((846_{(\mu l/rev)} * 180_{(rev/min)})/60_{(sec)})/1000_{(\mu l)}/1_{(mL)} = 2.538_{(mL/sec)}$$

For a 10 msec time point, loop 2 would be used. Loop 2 has an approximate reaction loop volume of 35 μl (see Table I). We also use the volume delivered from the syringes per revolution of the motor, measured as described under section VI., C. Syringe Volume Delivered per Revolution. A standard value is 846 μl per revolution. These volumes may vary, so calculations should be based upon the calibration of your instrument. The flow rate is calculated according to the following formula:

$$\text{FlowRate}_{(mL/sec)} = \text{loop volume}_{(\mu l)} / \text{reaction time}_{(sec)} * 1000_{(uL)}/1_{(mL)}$$

$$\text{Example: FlowRate}_{(mL/sec)} = 35_{(\mu l)}/ 0.01_{(sec)} / 1000_{(uL)}/1_{(mL)} = 3.5_{(mL/sec)}$$

Next enter the drive distance in μl. The terminal will calculate the necessary steps to move. Multiple cycles can be used with a “wait time” between cycles. Once these values have been entered, press GO on the screen to initiate the reaction.

VIII. PRECAUTIONS

In performing an experiment, there are only a few experimentally fatal mistakes that you might make. The first and most serious in the short run would be to apply a vacuum to the exit line and attempting to flush while the load valves are positioned toward the samples--- you would then very rapidly suck your entire sample right down the drain. This is a mistake very few people have ever made and no one has ever made more than once!

The only other caution is that you should always be careful to note that the valves are in the correct position before pressing GO. The danger here is that the motor is powerful enough to drive the syringes forward regardless of whether or not the valves are in the correct position. This generally will result in solution being forced back up through the valve. The solution then sits in the stem of the valve and causes corrosion which will shorten the lifetime of the valve.

IX. MAINTENANCE

Every month, a thin film of light weight oil (such as 3 in 1 oil) should be placed on the ball screw and on the vertical support rods.

The sample contacts only Teflon, glass and PEEK, all of which are resistant to organic solvents and oxidizing acids. Vapors from acid might cause some rusting of the drive syringe plungers if left in the syringes for long periods (days). Keep the apparatus cleaned and flushed with water in between experiments.

If you find that it is necessary to change any of the delay lines or any of the reaction loops, the apparatus can be disassembled by removing the plastic syringe box and then removing the screws on the plates on the back of the box in order to get access to the plumbing. Connections with new tubing can be made using connectors available from KinTek. Do not screw the connectors in too tight! Finger tight connection is sufficient to withstand 100 pounds per square inch of pressure. There is a wrench provided in a tool kit available from KinTek that can be used to ease the replacement of tubing and connectors. If you over tighten the screws, you will cause the ferrules to be constricted so that the flow will be retarded through the connection leading to excess back pressure.

Although individual delay lines and sample loops can be changed quite easily, we do not recommend that you undertake a complete overhaul of the apparatus or do anything with the 8-way valve. The 8 way valve has been polished and adjusted quite precisely in order to operate smoothly. If you have any problems at all with the 8 way valve, the unit should be returned for evaluation.

The sample loading valves and the syringe loading valves can be easily replaced and are available from KinTek. Alternatively, the entire unit can be returned to KinTek for repair or refurbish.

A water bath is used to control the temperature. You should use a circulating water bath that allows you to control the flow to prevent excess pressure buildup in the quench-flow box. It is highly recommended that the water in the circulating bath be changed every 2 weeks at a minimum, the unit should not be stored with water in the cooling chamber.

X. APPENDIX A. CONDITIONS FOR TURBULENT FLOW

Fast flow rates need to be maintained to obtain efficient mixing. Turbulent flow through a tube can be predicted by calculation of the Reynolds Number, which is a dimensionless parameter dependent upon the flow rate, V, the kinematic viscosity, ν, and the diameter of the tube, d:

$R = V*d/\nu$

Turbulent flow occurs when the Reynolds number exceeds 2000 (5, 6). Thus, for the 0.08 cm diameter tubing in the KinTek Quench Flow, and a kinematic viscosity of 0.01 stokes (cm-cm/sec), a linear flow rate of 2.5 m/sec must be maintained. Using the 5 ml drive syringes, a linear flow rate of 5.0 m/sec is achieved at a drive rate of 180 rpm, the minimum speed recommended. This provides a Reynolds Number of 4000.

XI. APPENDIX B. TEST REACTIONS

The instrument has been checked many times to confirm that it mixes and quenches reactions according to specifications. However, you can run a test reaction to check that you are using the instrument properly and that it is performing up to specifications. To obtain suggested test reactions please contact KinTek Corporation or the support section of our web site at www.kintekcorp.com.

XII. APPENDIX C. REFERENCES

1. Johnson, K. A. (1986) Rapid Kinetic Analysis of Mechanochemical ATPases. Meth. Enzymol. 134, 677-705.

2. Johnson, K. A. (1992) Transient State Kinetic Analysis of Enzyme Reaction Pathways. The Enzymes, XX, 1-61

3. Johnson, K. A. (1995) Rapid Quench Kinetic Analysis of Polymerases, ATPases and Enzyme Intermediates. Methods in Enzymology 249, 38-61.

4. Froehlich, J. P., Sullivan, J. V., and Berger, R. L. (1976) A chemical quenching apparatus for studying rapid reactions. Anal. Biochem. 73, 331-341.

5. Barman, T. E., and Gutfreund, H. (1964) in Rapid Mixing and Sampling Techniques in Biochemistry. (Chance, B., Eisenhardt, R.H., Gibson, Q.H., Lonberg-Holm, K.K., eds.) p. 339. Academic Press, New York.

6. Berger, R. L. (1964) in Rapid Mixing and Sampling Techniques in Biochemistry. (Chance, B., Eisenhardt, R. H., Gibson, Q. H., Lonberg-Holm, K.K., eds.) p. 363. Academic Press, New York.

XIII. APPENDIX D. PARTS AND ACCESSORIES

Syringes, valves, tubing and connectors: Syringes and valves, tubing, and tubing connectors can be directly ordered from KinTek Corporation:

500 West Sycamore Road, Snow Shoe, PA 16874
PH: [814] 387- 4678 • FAX: [814] 387- 4974
www.kintek-corp.com

Vacuum pump: You will need a vacuum source for flushing the system between runs. If one is not available, we recommend a small diaphragm pump, connected to a suction flask to receive the flush solutions. One that works is listed below.

KNF™ Neuberger LABOPORT™ Mini Diaphragm Vacuum Pump:

Fisher Scientific Cat. No. 13-880-904
Millipore Sigma SKU No. Z288292

Temperature Probe: A temperature probe can be installed by replacing the plug in the right side of the plastic box with a compression fitting and probe. Alternatively, the 1/8 inch (3.17 mm) plug can be replaced with your temperature probe.

Circulating Water Bath: A circulating water bath for temperature control may be connected at the two water ports of the RQF-3 chamber. The ThermoFisher TF900 or a similar temperature controlled water bath will suffice.

Site: http://www.thermofisher.com Catalog number: TF900

XIV. APPENDIX E. QUENCH FLOW TUBING LENGTHS

This list provides the cut length for various segments of tubing used to construct the quench-flow. Tubing lengths can be ordered from the factory with fittings installed. Call for pricing.

Reaction Loops:

Loop Number	Length
1	0
2	3 cm
3	8 cm
4	15 cm
5	23 cm
6	31 cm
7	40 cm
8	5 cm

Syringe Valve Lines:
2 @ 5 cm
1 @ 7 cm

Sample Load Lines:
2 @ 5 cm
2 @ 11.5 cm

Quench Line:
1 @ 14 cm

Flush Line (one connector only):
2 @ 12 cm

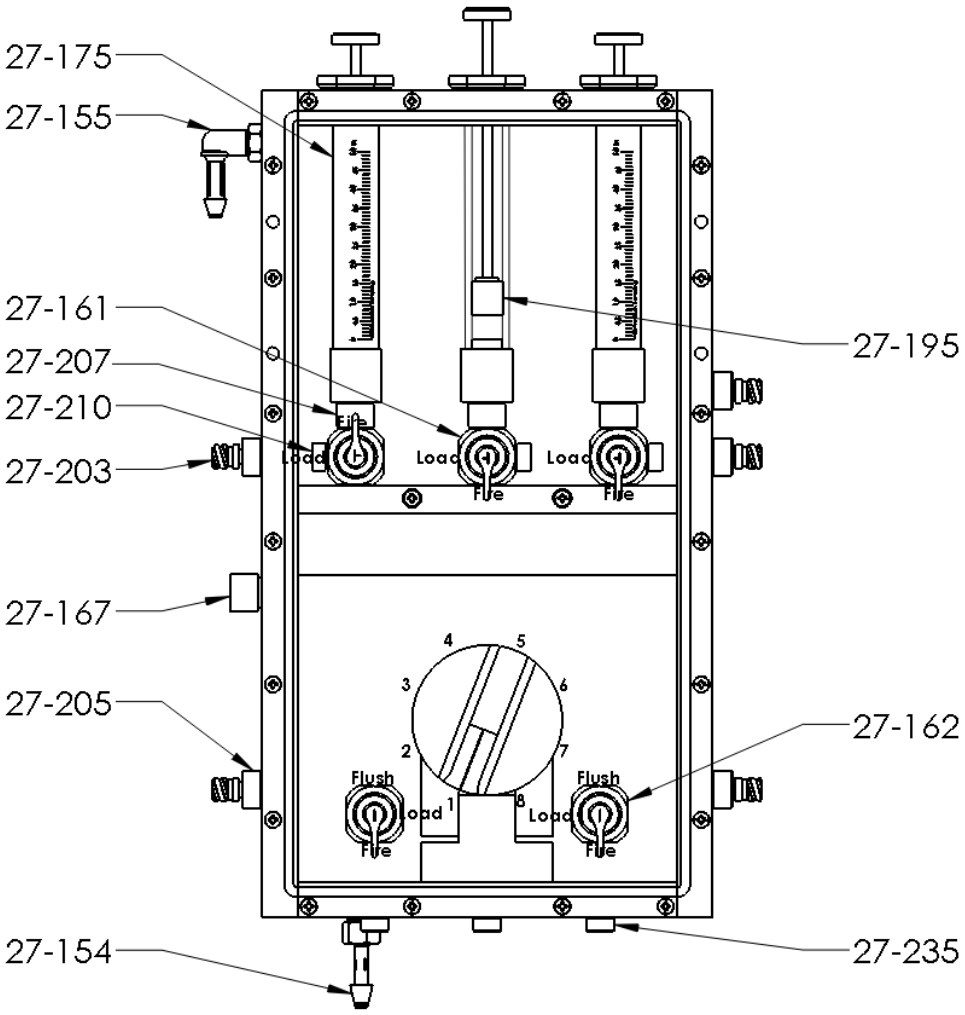
Exit Loop (one connector only):
1 @ 20 cm

Sample Loops:
0.5 mm tubing diameter
2 @ 5 cm

XV. APPENDIX F. REPLACEMENT PART NUMBERS AND DESCRIPTIONS

27-001	Replacement Syringe/Valve Chamber for RQF-3
27-002	Additional Syringe Chamber for RQF-3
27-150	Sample Load Loops, 15μl (not shown)
27-154	Circulating Water Fitting
27-155	Circulating Water Fitting 90°
27-161	3-way Upper Drive Syringe Loading Valve
27-162	2-way Lower Sample Loading Valve
27-167	Temperature Probe Holder/Plug Assembly
27-175	Syringes for RQF, 5ml
27-185	Plunger Assembly for 5ml Syringe
27-203	Luer Lock Syringe Connecting Adapters
27-205	Side Panel Mounts (screw into box sides)
27-207	Syringe/Upper Valve Adapter
27-210	Minstac/Valve Adapters (screw into upper valves)
27-235	Exit Loop Compression Fittings
27-300	8-way Reaction Loop Valve
27-400	Micro-Tubing Connector Kit, Tools, 25 Connectors, Tubing (not shown)
27-405	Micro-Tubing Wrench (not shown)

Please Call Factory at: 814-387-4678
or see our web site: www.kintekcorp.com for pricing and delivery.



PART NUMBER	DESCRIPTION	QTY.
27-155	90° Nylon Tube Fitting	1
27-154	Nylon Tube Fitting	1
27-205	Panel Mount	5
27-167	Temperature Probe Fitting	1
27-175	Syringe for RQF, 5ml	3
27-161	3 Way Upper Valve	3
27-207	Syringe Connect Adapter	3
27-210	Minstac Adapter	6
27-162	2 Way Lower Valve	2
27-235	Compression Fitting	3
27-203	Luer Lock Syringe Adapter	5
27-195	Plunger Tip	3

Figure 4. ***Part Designation of Quench-Flow System.*** **The part numbers are shown in this figure to assist with the ordering of replacement parts or systems. Please call for pricing.**