KinTek Corporation

Chemical-Quench-Flow
Servo-Motor
Models RQF-3 and RQF-4

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.

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I. INSTALLATION OF THE INSTRUMENT

1. There is one cable connection which goes from the instrument to the computer terminal.

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 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

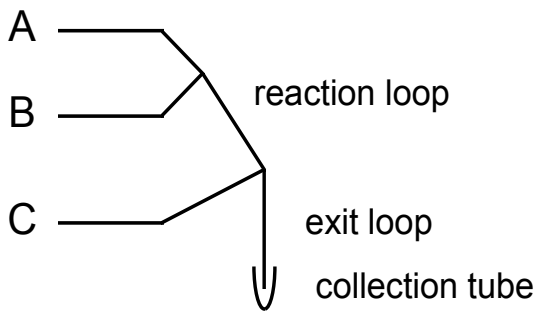
A. 3 Syringe Operation

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 (see Figure 1a), and the rate of flow, F:

$$t = V/F(\mu l)/(\mu l/msec)$$

To vary the reaction time, you can vary the volume of the reaction loop or the rate of flow. There are practical limits on range over which either of these can be varied. The KinTek Quench-Flow is supplied with 7 reaction loops varying from approximately 16 μl to 200 μl, providing reaction times from approximately 1-80 msec.

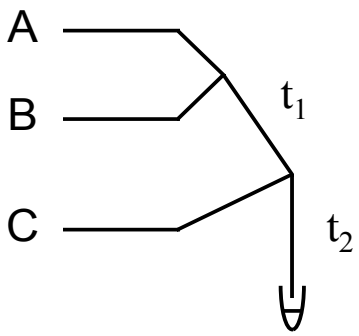
Reactants A and B are first mixed and the reaction proceeds while they flow through the variable length reaction loop. The reaction is stopped by mixing with a quench solution contained in syringe C. The mixture is then expelled into a collection tube.



To achieve reaction times greater than 80 msec, a push-pause-push mode is used; the first push mixes the reactants 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 with the quench solution 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, select option 8 from the main menu and press <ENTER> when "Use Second Delay?" comes up. A second reaction time can then be entered into the computer under option 1 "Quench Flow Run". The motor will then pause for the appropriate time to allow the reaction time, t_2, prior to expelling the reactants. Our "2nd quench delay" is the time, t_2.



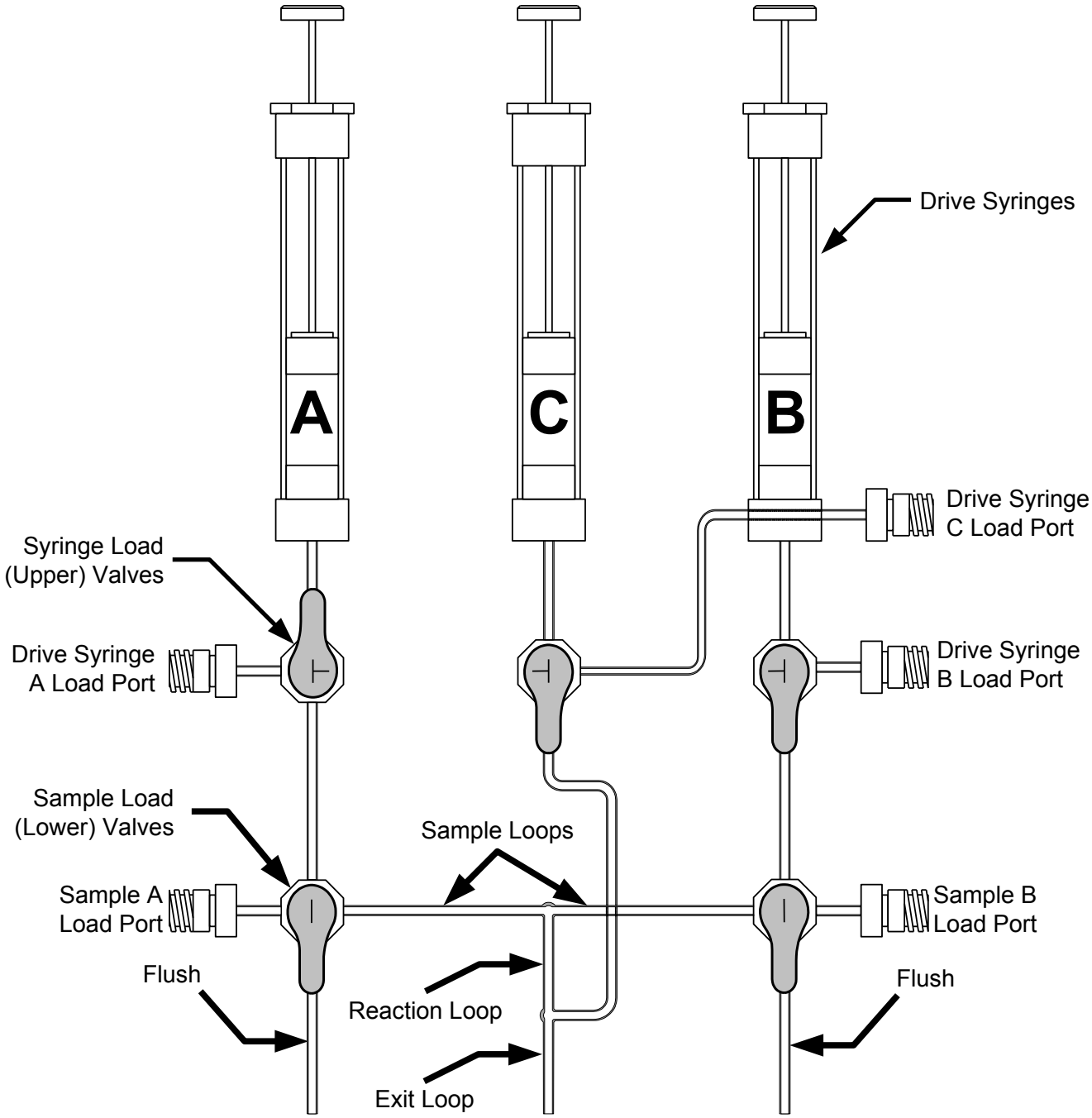


Figure 1a. ***Schematic of the 3 Syringe Quench-Flow Valve System.*** **The valves are shown in the proper position to FIRE a shot.**

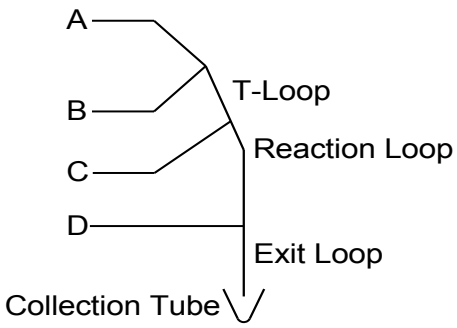
B. 4 Syringe Operation

In a four syringe chemical quench-flow experiment, a reaction is initiated by the mixing of two reactants (Samples A+B) into a T-Loop. The reaction pause time is set by the user. The mixed reactants (A+B) flow into a reaction loop along with a third reactant (C). The loop will correspond with the amount of time user specifies for the second push. The final push mixes reactants(A+B+C) with a quench solution to stop the reaction and expels solution for analysis. The time of the reaction, t, is thus determined by the volume of the reaction loop, V (see *Figure-1b*), and the rate of flow, F:

$$t = V/F \ (\mu l)/(\mu l/msec)$$

To vary the reaction time, you can vary the volume of the reaction loop or the rate of flow. There are practical limits on range over which either of these can be varied. The KinTek Quench-Flow is supplied with 7 reactions loops varying from approximately 16 μl to 200 μl, providing reaction times from 2-47 msec.

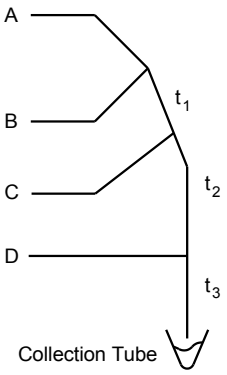
Reactants A and B are first mixed and paused. The reaction proceeds with Reactant C mixing with A+B thru a variable length reaction loop. The reaction A+B+C is stopped by mixing with a quench solution contained in syringe D. The mixture is then expelled into a collection tube.



To achieve reaction times greater than 47 msec, a push-pause-push-pause-push mode is used; the first push mixes the reactants into the t-loop, the apparatus then pauses for a defined period to allow the reaction to proceed, then a second push moves the solutions into the reaction loop and then pauses. The third push expels the reactants, mixing with the quench solution 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 tell you which loop to use and automatically set the appropriate flow rate.

A third 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. The time for the third reaction, t_3, can be entered into the computer under Option "8" as described in section III, below. The motor will then pause for the appropriate time to allow the reaction time, t_3, prior to expelling the reactants. Our "3rd" quench delay is the time, t_3.



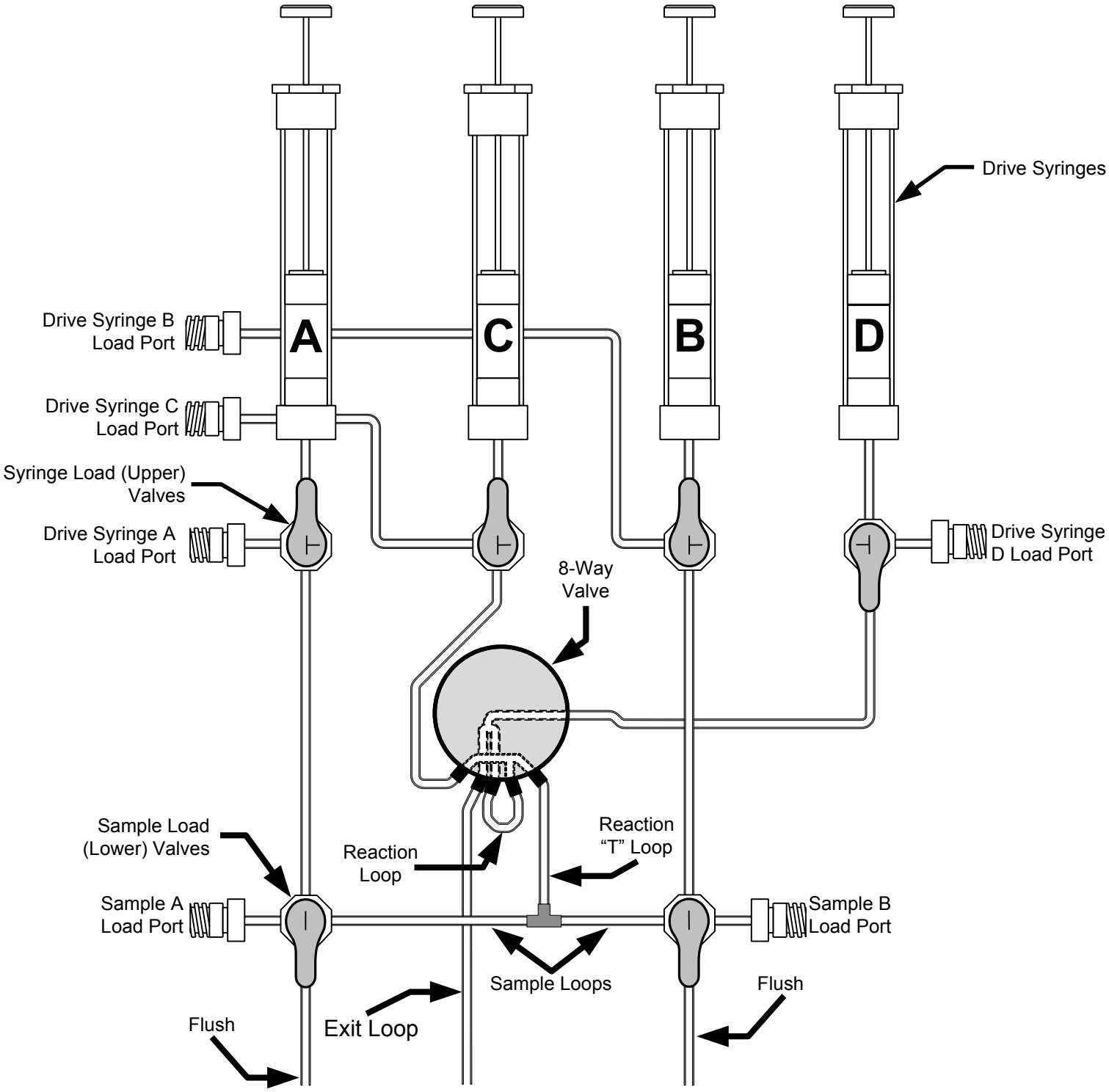


Figure 1b. ***Schematic of the 4 Syringe Quench-Flow Valve System.*** **The valves are shown in the proper position to FIRE a shot.**

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 box. If you engage one of the limit switches, simply reverse the direction of the motor to back it away from the end as described below under option 2…ADJUST POSITION or under option 7…GO TO HOME POSITION.

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, 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.

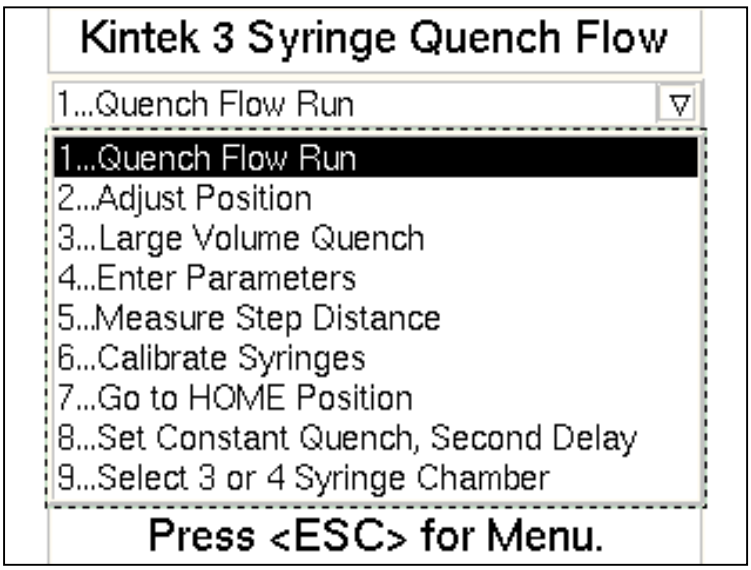
Specific instructions for each option of the computer program are presented below. In general, runs can be aborted by pressing the escape key <ESC>. This will cause the program to exit to the main menu.

The Kintek Quench Flow terminal keypad is shown below. The specific functions of the keys and how they pertain to the program are outlined in the instructions for each option of the computer program.

The KinTek Quench Flow terminal key pad.

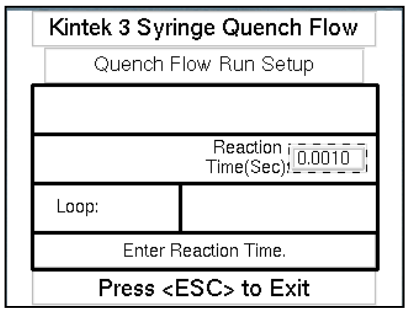
A. 3 Syringe Operation:

When the system is first turned on it will display “Motor initializing...” The syringe platform will find the upper limit switch and move back down one revolution. This position becomes “home”. After the syringe platform reaches the “home” position the terminal will proceed to the main menu.

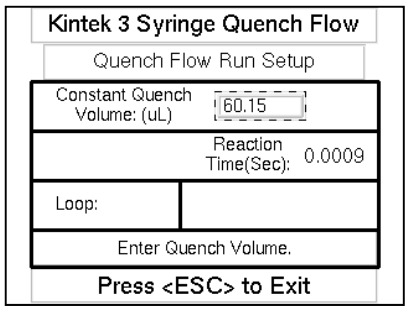


1. QUENCH-FLOW RUN

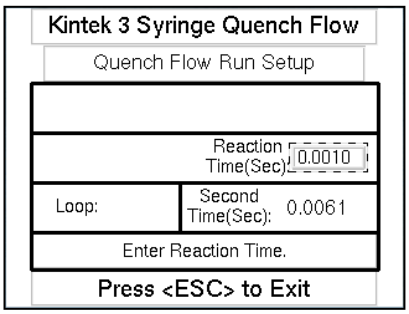
After calibration parameters have been entered, this option will be used to perform a normal quench-flow experiment. Depending on the options chosen for constant quench and reaction delay (See Option 8), one of the following screens will appear:



Normal operation



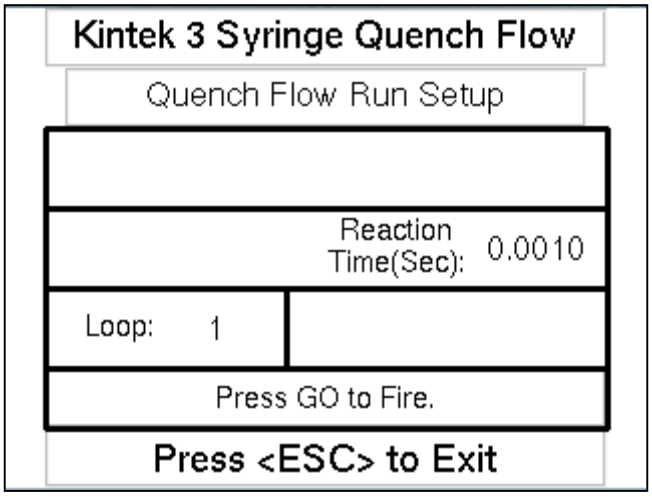
Constant Quench Used



Second Delay Used

The terminal computes the minimum reaction times or volume and enters these values in the fields automatically. The minimum values shown depend on your calibration values (See 4…Enter Parameters) and the maximum speed of the motor. If a larger quench volume or longer times are desired, press Enter to focus on the field and enter the desired value.

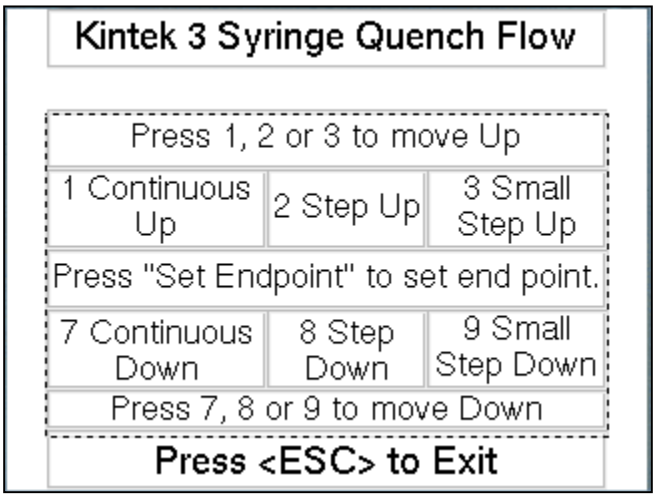
Once the desired values are entered, the terminal will display the required loop for the desired time and prompt for “GO” to be pressed.



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.

2. ADJUST POSITION

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



There are 3 modes of operation in the adjust motor mode.

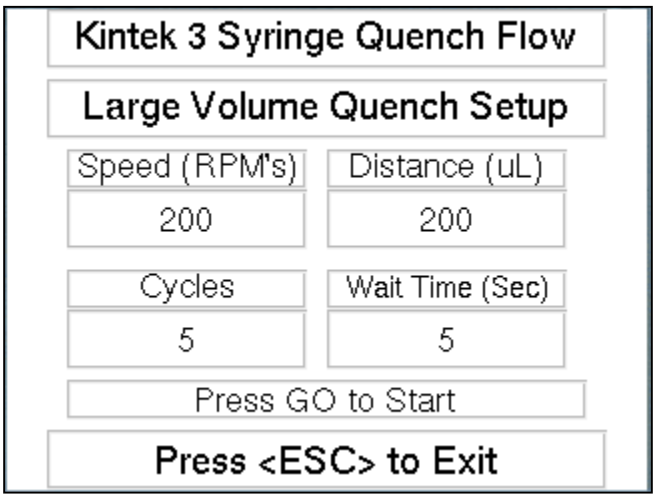
i. Continuous mode
ii. Press “1” or “7” to move the platform up or down continuously. The platform will move for as long as the user presses the keypad.
iii. Step mode
iv. Press “2” or “8” to move the platform up or down in large steps. For each press of the keypad, the platform will move a finite distance up or down.
v. Small Step mode
vi. Press “3” or “9” to move the platform up or down in small steps. For each press of the keypad, the platform will move in small steps up or down. This mode is used for fine adjustments of the platform.

The endpoint is also set from this screen. To set the endpoint, move the platform to the desired endpoint position and press the <Set Endpoint> key on the keypad. The value of the new end point (in steps from the home point) will automatically be saved in Option 4 and the following message will appear:

Press <ENTER> to return to the motor adjust screen.
Pressing <ESC> from the motor adjust screen will return the terminal to the main menu.

3. SPEED & DISTANCE (LARGE VOLUME QUENCH)

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



Speed (RPM's) – Enter the desired motor speed in RPM's (revolutions per minute). The maximum speed is 1600 RPM's and the minimum set speed is 0.01 RPM (entered values less than 0.01 will automatically default to 0.01). A more typical speed for the quench flow run is 200 RPM's. See Section V and Appendix A for a discussion of the relationship between motor speed and flow rates.

Distance (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 entered in the parameters screen.

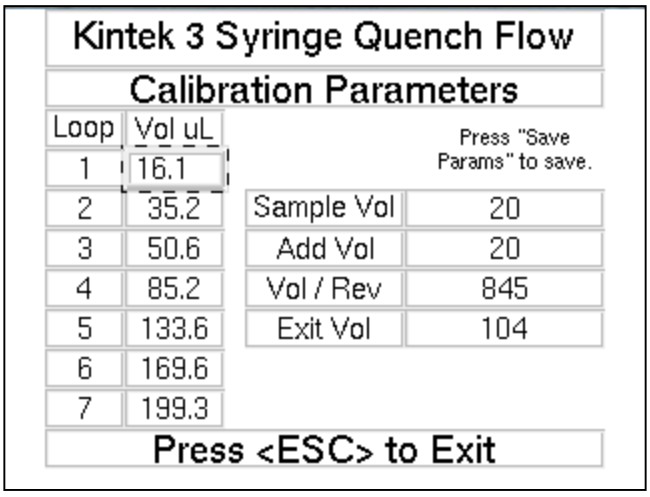
Cycles – Enter the number of times you want a push-pause-push cycle to be repeated. If "1" is entered here, the terminal will not prompt for a "Wait Time", it will simply display "Press GO to Start".

Wait Time (Sec) – Enter the time delay between push cycles, in seconds.

Once these parameters have been entered, press the <GO> key on the keypad to start the reaction. Press <ESC> to abort the run and return to the Large Volume Quench Setup screen. Press <ESC> from the Large Volume Quench Setup screen to return to the main menu.

4. ENTER PARAMETERS

This option is used to enter the calibration parameters, the 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). 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 PARAMS" button or they will revert to their previously saved values. Once saved they are stored in the hand-held terminal and it is not necessary to modify them or re-enter them unless a change has been made to the syringe chamber which affects the system calibration. 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.



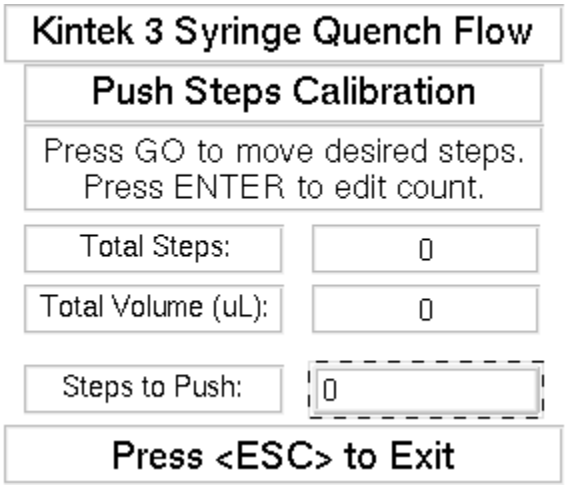
5. MEASURE LOOP DISTANCE

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 LOOPS and DELAY LINE as described below 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 LOOPS and DELAY LINES to bring the buffer just up to the tip of the EXIT LOOP. 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 Option 6)
SPR = Steps per Revolution = 8192

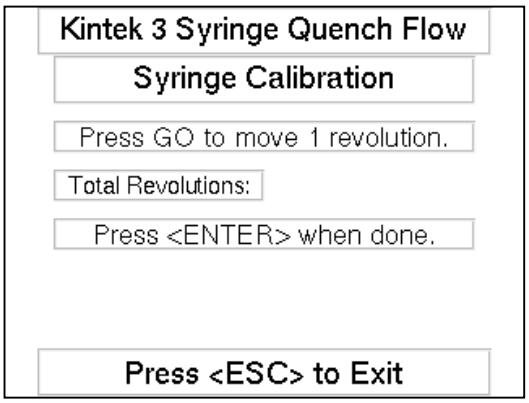
Enter the distance in steps desired or press “RETURN” to use the default (10). See Table I for approximate distances for each loop. The motor will immediately begin moving slowly and then stop.

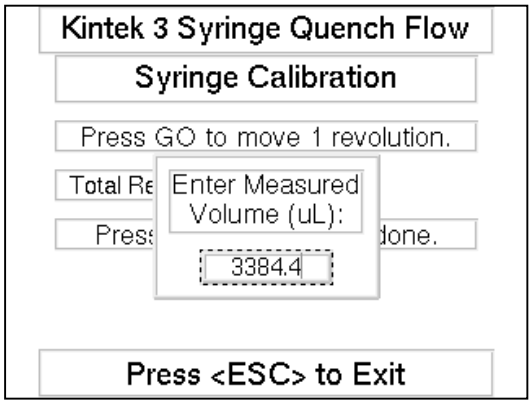


Enter “0” to reset the step count for the next loop, or press <ESC> to exit to the main menu.

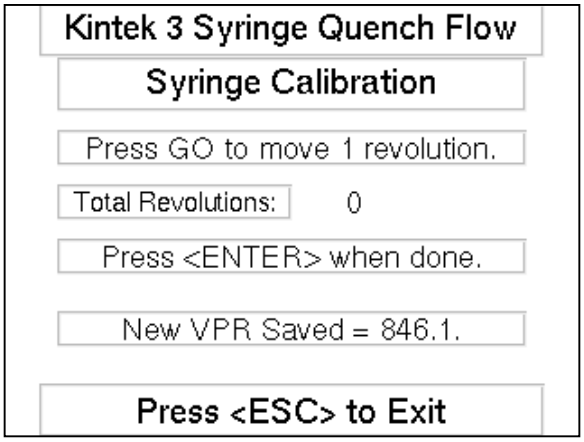
6. CALIBRATE SYRINGES

This option allows a simple calibration of the volume delivered from the two drive syringes per revolution of the motor. This volume should be approximately 845 μl. First load the two buffer drive syringes with water, removing all air bubbles. DO NOT load solution into the quench syringe (the middle syringe). Place the valves in the FIRE positions and then advance the drive plate to expel solution from the exit line until all bubbles are removed (do this using option 2 from the main menu). This portion of the program simply causes the motor to advance one revolution each time <GO> is pressed. Measure the volume delivered by weight to establish the volume per revolution. This parameter will automatically be entered in the calibration screen under Option 4. It is recommended to push several revolutions into a weighed vial, and then weigh it again to get a precise measurement over multiple pushes. After each push, the display will show the number of revolutions. Once 3 to 4 revolutions are complete, press <ENTER> and the terminal will prompt for the measured volume.





Once the measured volume has been entered, the terminal will calculate and save the new Volume Per Revolution (VPR) based on the number of revolutions and entered volume delivered.



It is not necessary to enter this value under Option 4 manually.

7. GO TO HOME POSITION

This option 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 to the HOME position. Subsequently, the motor will go directly to the HOME position and the following message will be displayed:

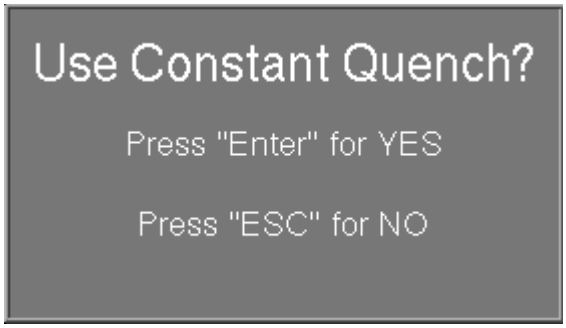
Once the platform is home, the terminal will return to the main menu.

8. SET CONST QUENCH, SECOND DELAY

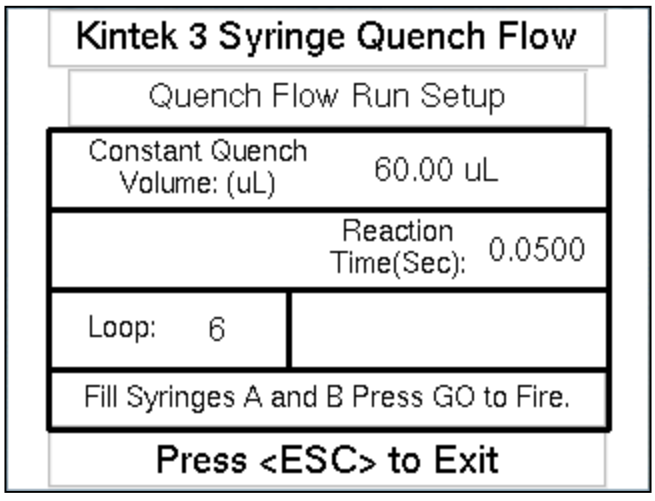
This menu item allows control over two additional features of the quench-flow operation.

i. Constant Quench Volume

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 delay line is being filled. In order to obtain a constant quench volume, the syringe drive motor will back up before each run after you enter the reaction time. The quench volume delivered from the quench syringe will then be the same for each reaction loop, minimally, equal to the volume normally delivered through loop 1. **You must then manually refill the buffer drive syringes to bring their plungers up to meet the drive platform**. To use this feature, answer yes (press <ENTER>) in response to the question:

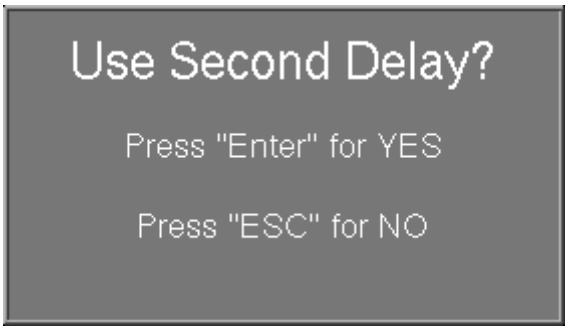


During a quench flow run, the terminal will prompt for the buffer syringes to be filled before prompting to fire:

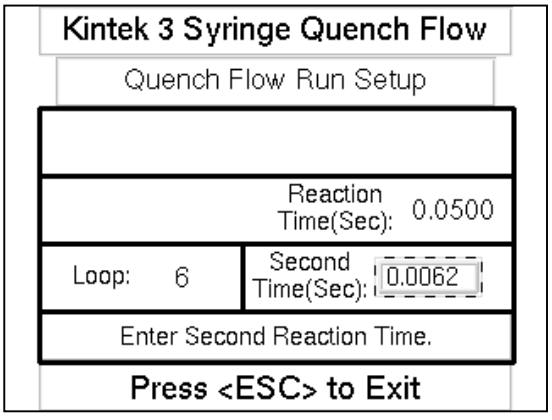


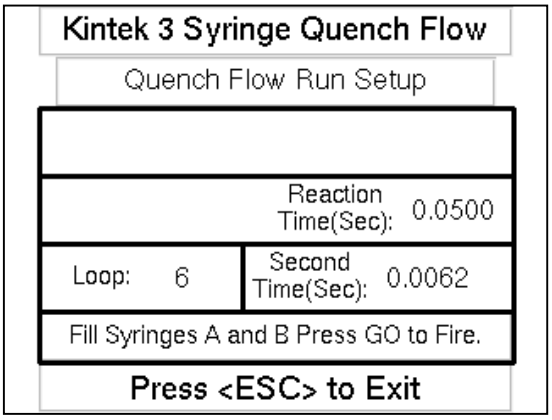
ii. 2nd Quench Delay

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 for example where you may quench with an excess of unlabelled 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. To use this feature, you must calibrate the volume of the exit loop (see instructions) and enter the value under menu item 4. Then when you run the program, answer yes (press <ENTER>) in response to the question:



During a quench flow run, the terminal will prompt for a second delay time and prompt for buffer syringes to be loaded, just as if using the minimum quench solution.



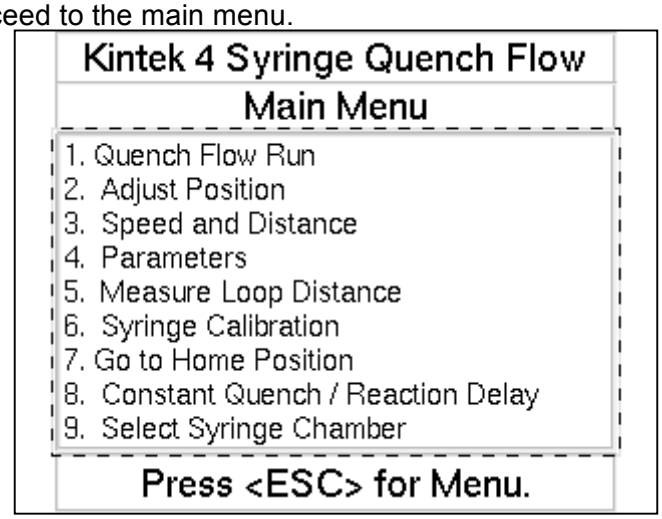


9. SELECT SYRINGE CHAMBER

Some labs may have a KinTek 3 Syringe, and a KinTek 4 Syringe chamber. This option alleviates the need to update the entire program and re-enter calibration parameters based on the chamber being used. Choose 3 or 4 syringe chamber and all calibration parameters will be automatically updated. There are also selections for Freeze Quench operation, these files share a common calibration file with the 3 syringe chamber so the user must change those parameters when they switch chambers,

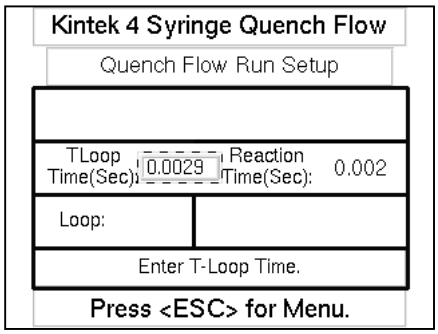
B. 4 Syringe operation:

When the system is first turned on it will display “Motor initializing...” The syringe platform will find the upper limit switch and move back down one revolution. This position becomes “home”. After the syringe platform reaches the “home” position the terminal will proceed to the main menu.

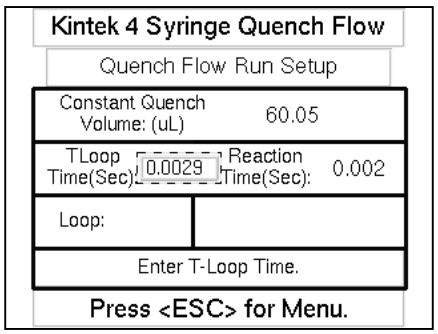


1. QUENCH-FLOW RUN

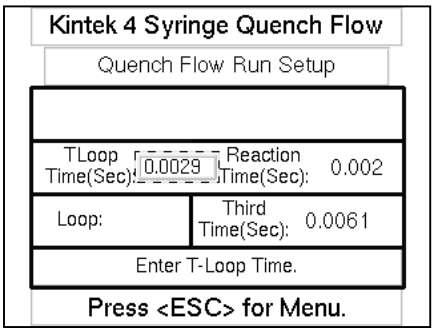
After calibration parameters have been entered, this option will be used to perform a normal quench-flow experiment. Depending on the options chosen for constant quench and reaction delay (See Option 8), one of the following screens will appear:



Normal operation



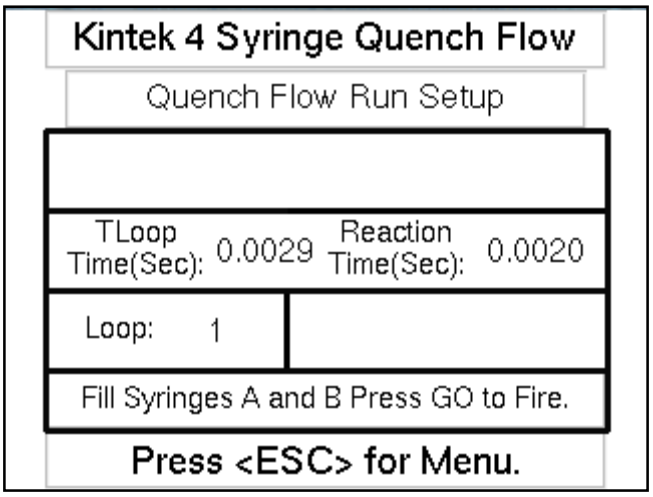
Constant Quench Used



Second Delay Used

The terminal computes the minimum reaction times or volume and enters these values in the fields automatically. The minimum values shown depend on your calibration values (See 4…Enter Parameters) and the maximum speed of the motor. If a larger quench volume or longer times are desired, press Enter to focus on the field and enter the desired value.

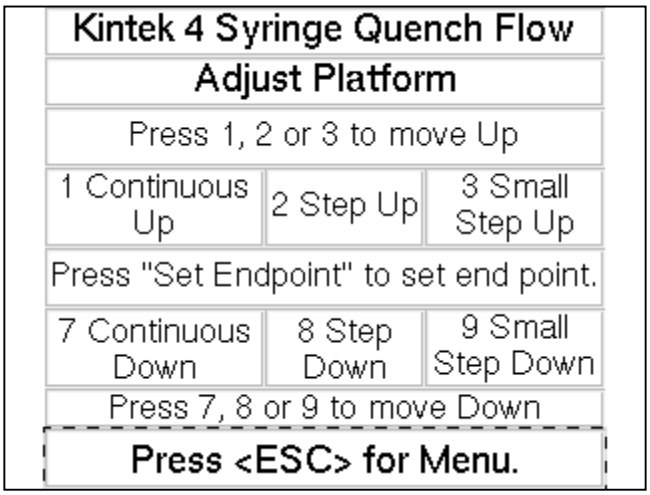
Once the desired values are entered, the terminal will display the required loop for the desired time. It will also prompt for specific syringes to be filled depending on normal operation, constant quench volume, or second delay.



Once the prompted syringes are filled, press “GO” to initiate 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.

2. ADJUST POSITION

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



There are 3 modes of operation in the adjust motor mode.

i. Continuous mode
Press “1” or “7” to move the platform up or down continuously. The platform will move for as long as the user presses the keypad.

ii. Step mode
Press “2” or “8” to move the platform up or down in large steps. For each press of the keypad, the platform will move a finite distance up or down.

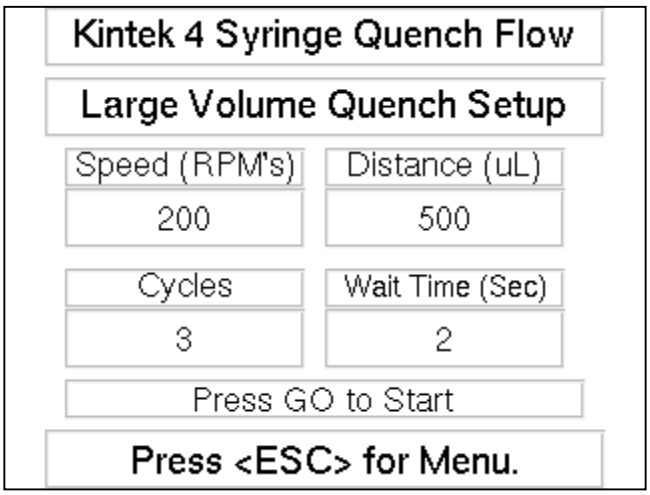
iii. Small Step mode
Press “3” or “9” to move the platform up or down in small steps. For each press of the keypad, the platform will move in small steps up or down. This mode is used for fine adjustments of the platform.

The endpoint is also set from this screen. To set the endpoint, move the platform to the desired endpoint position and press the <Set Endpoint> key on the keypad. The value of the new end point (in steps from the home point) will automatically be saved in Option 4 and the following message will appear:

Press <ENTER> to return to the motor adjust screen.
Pressing <ESC> from the motor adjust screen will return the terminal to the main menu.

3. LARGE VOLUME QUENCH

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



Speed (RPM's) – Enter the desired motor speed in RPM's (revolutions per minute). The maximum speed is 1600 RPM's and the minimum set speed is 0.01 RPM (entered values less than 0.01 will automatically default to 0.01). A more typical speed for the quench flow run is 200 RPM's. See Section V and Appendix A for a discussion of the relationship between motor speed and flow rates.

Distance (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 entered in the parameters screen.

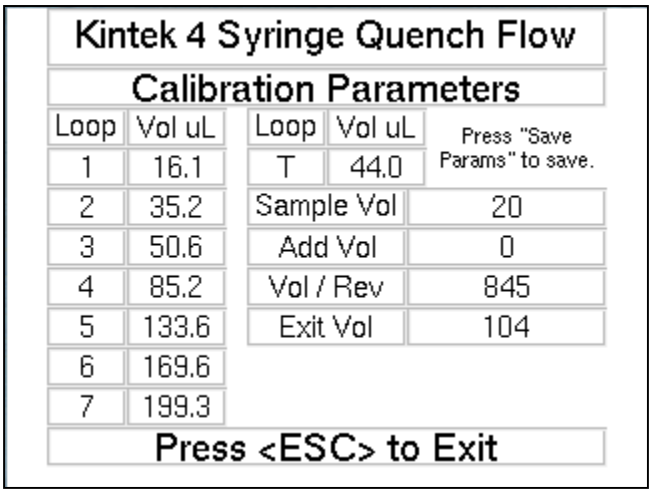
Cycles – Enter the number of times you want a push-pause-push cycle to be repeated. If "1" is entered here, the terminal will not prompt for a "Wait Time", it will simply display "Press GO to Start".

Wait Time (Sec) – Enter the time delay between push cycles, in seconds.

Once these parameters have been entered, press the <GO> key on the keypad to start the reaction. Press <ESC> to abort the run and return to the Large Volume Quench Setup screen. Press <ESC> from the Large Volume Quench Setup screen to return to the main menu.

4. ENTER PARAMETERS

This option is used to enter the calibration parameters, the 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). 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, they are saved in the hand-held terminal and it is not necessary to modify them or re-enter them unless a change has been made to the syringe chamber. Syringe chamber calibration is covered in Section VI of this manual.



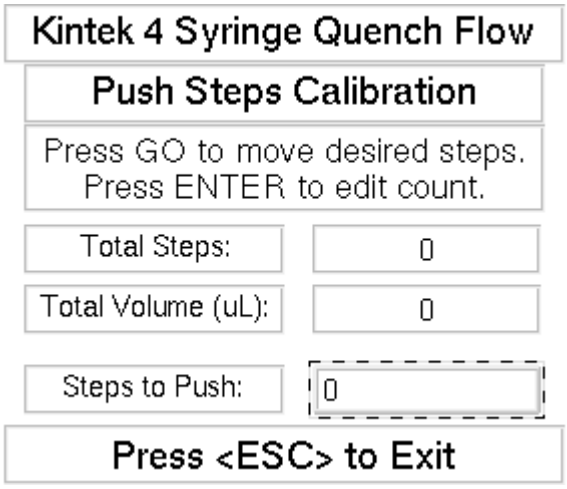
5. MEASURE LOOP DISTANCE

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 LOOPS and DELAY LINE as described below 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 LOOPS and DELAY LINES to bring the buffer just up to the tip of the EXIT LOOP. 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 Option 6)
SPR = Steps per Revolution = 8192

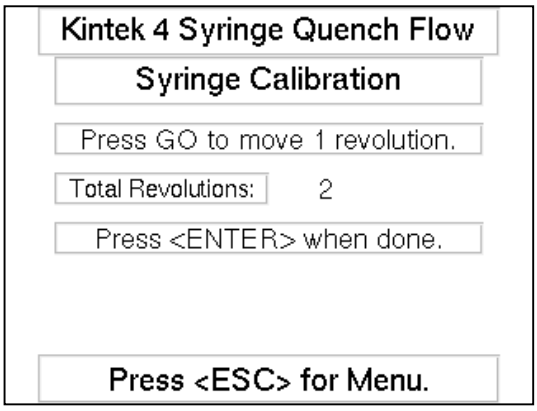
Enter the distance in steps desired or press “RETURN” to use the default (10). See Table Ib for approximate distances for each loop. The motor will immediately begin moving slowly and then stop.

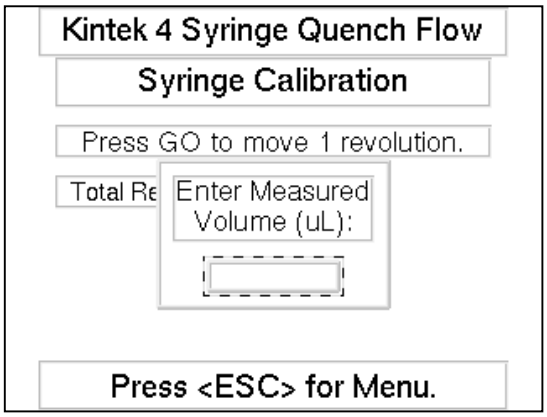


Enter “0” to reset the step count for the next loop, or press <ESC> to exit to the main menu.

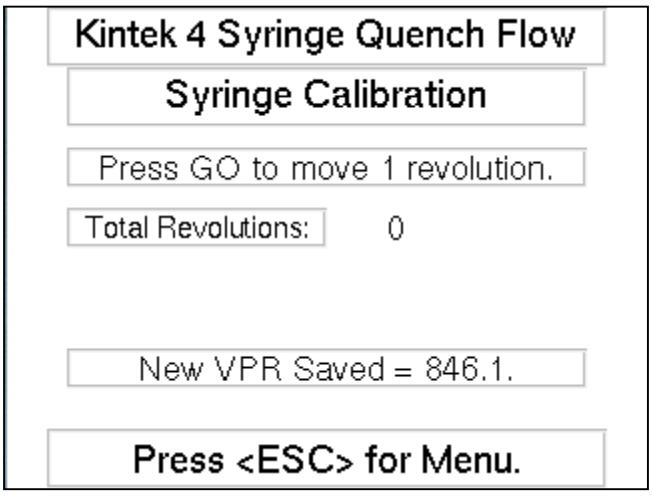
6. CALIBRATE SYRINGES

This option allows a simple calibration of the volume delivered from the two drive syringes per revolution of the motor. This volume should be approximately 846 μl. First load the two buffer drive syringes with water, removing all air bubbles. DO NOT load solution into the quench syringe (the middle syringe). Place the valves in the FIRE positions and then advance the drive plate to expel solution from the exit line until all bubbles are removed. This portion of the program simply causes the motor to advance one revolution each time <GO> is pressed. Measure the volume delivered by weight to establish the volume per revolution. This parameter will automatically be entered in the calibration screen under Option 4. It is recommended to push several revolutions into a weighed vial, and then weigh it again to get a precise measurement over multiple pushes. After each push, the display will show the number of revolutions. Once 3 to 5 revolutions are complete, press <ENTER> and the terminal will prompt for the measured volume.





Once the measured volume has been entered, the terminal will calculate and save the new Volume Per Revolution (VPR) based on the number of revolutions and entered volume delivered.



It is not necessary to enter this value under Option 4, it is saved automatically.

7. GO TO HOME POSITION

This option 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 to the HOME position. Subsequently, the motor will go directly to the HOME position and the following message will be displayed:

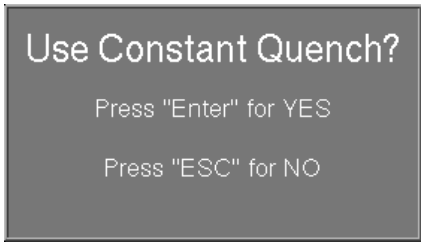
Once the platform is home, the terminal will return to the main menu.

8. SET CONST QUENCH, SECOND DELAY

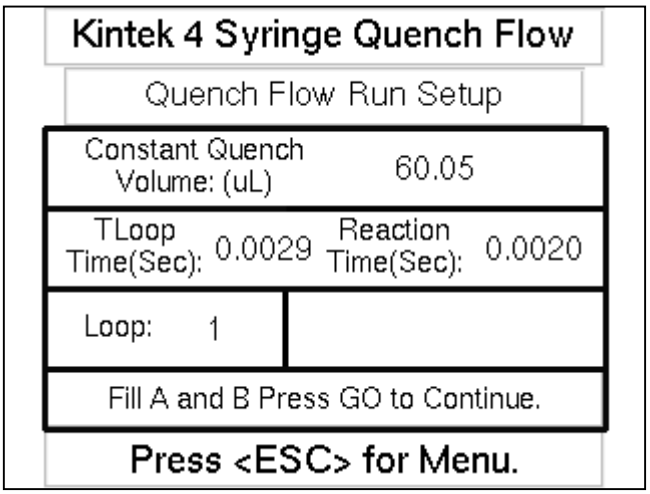
This menu item allows control over two additional features of the quench-flow operation.

i. Constant Quench Volume

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 delay line is being filled. In order to obtain a constant quench volume, the syringe drive motor will back up before each run after you enter the reaction time. The quench volume delivered from the quench syringe will then be the same for each reaction loop, minimally, equal to the volume normally delivered through loop 1. **You must then manually refill the buffer drive syringes to bring their plungers up to meet the drive platform**. To use this feature, answer yes (press <ENTER>) in response to the question:

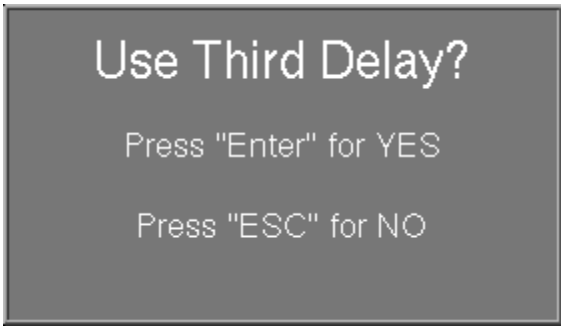


During a quench flow run, the terminal will prompt for the buffer syringes to be filled before prompting to fire:

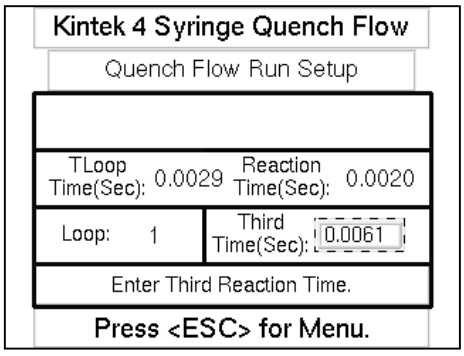


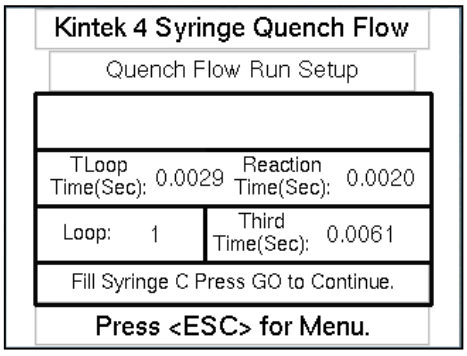
ii. 3rd Delay

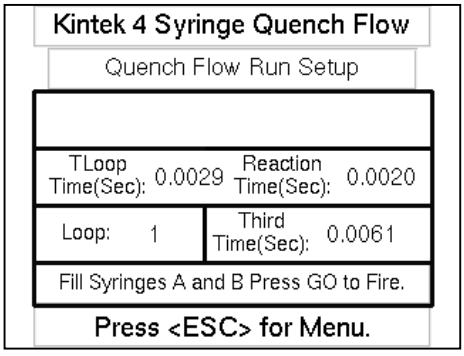
This option is useful when mixing multiple reactants in pre-determined time steps. The third delay is used to mix a fourth reactant from syringe D with A+B+C in the exit line. The third delay is the time that all four of these solutions will be allowed to react in the exit line prior to being expelled. These reactants may then be quenched by expelling into a quench solution held in a collection tube. To use this feature, you must calibrate the volume of the exit loop (see instructions) and enter the value under menu item 4. Then when you select option 8, answer yes (press <ENTER>) in response to the question:



During a quench flow run, the terminal will prompt for a third delay time and prompt for syringes to be loaded in a sequence that will allow the proper delivery of solutions to achieve the correct volumes on each push.







9. SELECT SYRINGE CHAMBER

Some labs may have a KinTek 3 Syringe, and a KinTek 4 Syringe chamber. This option alleviates the need to update the entire program and re-enter calibration parameters based on the chamber being used. Choose 3 or 4 syringe chamber and all calibration parameters will be automatically updated. There are also selections for Freeze Quench operation, these files share a common calibration file with the 3 syringe chamber so the user must change those parameters when they switch chambers.

IV. VALVE ARRANGEMENT

Figures 1a and 2 show 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 3-way 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 arrows 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 three-way sample valves, the outflow goes through the back of the valve and the single arrow points to the line which is 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 side port and turn the arrow 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 Tables I and II.

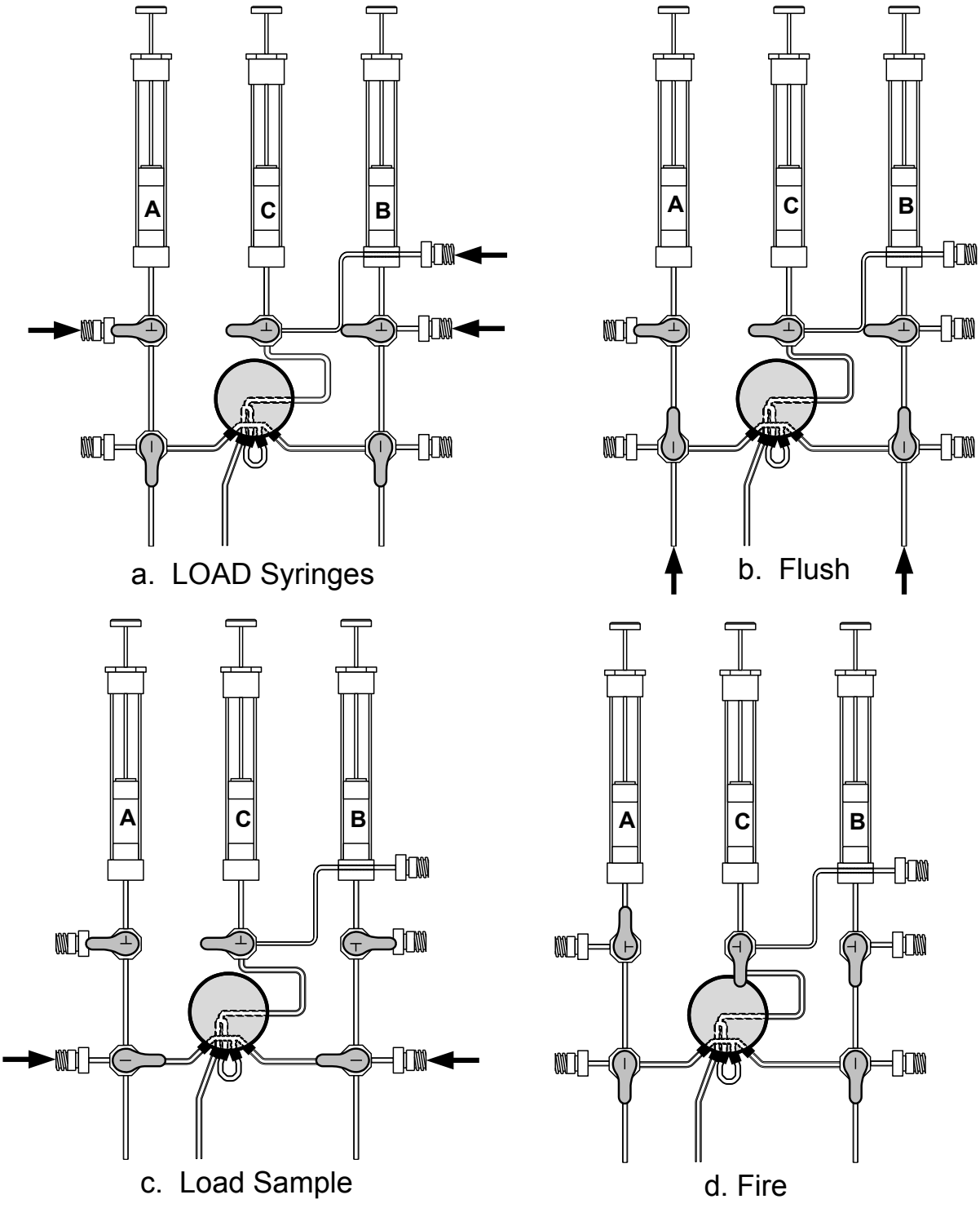


Figure 2 ***Valve Positions for Quench-Flow Operation.*** **The valve positions are shown for loading, firing and flushing sample lines. The small arrows indicate the ports used for loading or flushing.**

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 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 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 valves to the LOAD position and, while applying the suction, use a pipette to dribble buffer and then methanol into the syringe ports on the side of the apparatus. Continue sucking air until the syringe ports are dry.

C. The Load-Fire-Flush Cycle

To begin an experiment attach a 1 ml syringe containing your samples to each of the load lines. 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 metal connection on the eight-way valve. After loading each sample, turn the valve to the fire position so that now the valve is connected to the drive syringe line (Fig. 2c).

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 (Fig. 2d). Firing with the valves in the wrong position will force solution back up into the valve, causing corrosion of the valve.

4. Push the <GO> button on the keypad to start the reaction (see Section III). The servo motor will drive the syringes to force the reactants together through the reaction loop and into the 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 Loops – 3 Syringe

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

1. Calibration method using radioactive standard

   This is best done using 32P-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 32P-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. Load one of the sample lines with radioactive solution such that you fill the sample line, the reaction loop, and the exit line entirely and excess solution comes out of the exit line. You now have radioactive sample completely loading the lines as shown in Figure-3a.

   ii. By pushing buffer from the QUENCH loading syringe, 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 left then with radioactivity distributed as shown is Figure-3b.

   iii. By flushing with a solution from the other sample line (not used to load the radioactive sample), you then expel the contents of the reaction loop into a scintillation vial. This sample provides a measurement of the volume contained within that reaction loop. Now radioactive solution is contained only in the sample loop which was chosen for loading (Figure-3c).

   iv. By flushing with buffer from the BUFFER loading syringe, 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 loops, 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 syringe, 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 is Figure 3b.

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

iv. By flushing with buffer from the BUFFER loading syringe, 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 loops, 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:

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

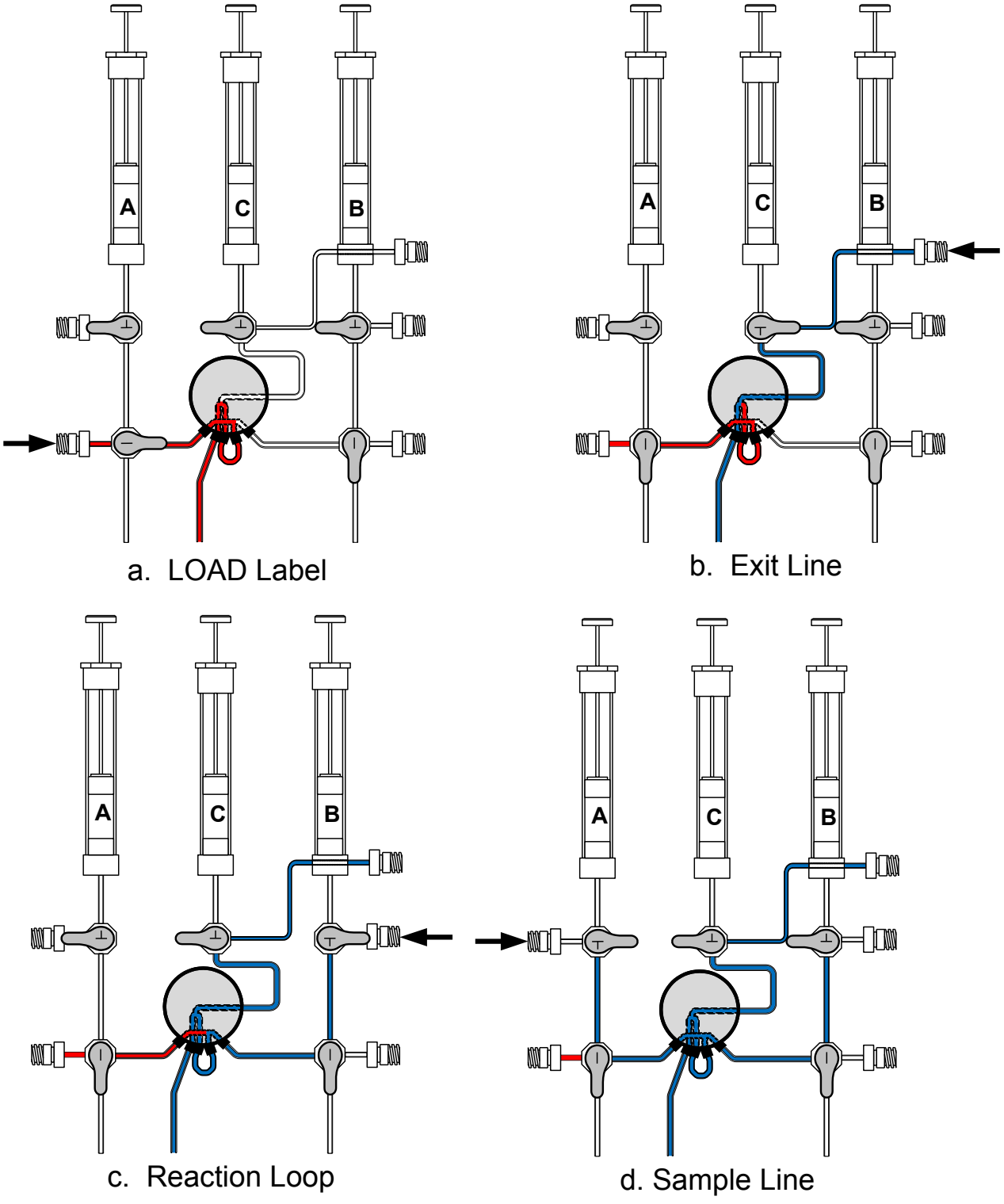


Figure 3. ***Calibration of Sample and Reaction Loops.*** **The method for calibrating the sample loops and reaction loops is outlined in this scheme. The sections of tubing containing radioactive sample are shown in red. The small arrows show the ports used to first load and then flush the various tubing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

B. Volumes of Reaction Loops and Sample Loops – 4 Syringe

The principal of operation for measuring the volume of each loop is shown in Figure-4. The volumes of the reaction loop lines and the sample load loops need to be calibrated precisely. This is done by loading a solution of radioactive or absorbance standard into each of the loops and then flushing to recover the solution contained within. By counting or measuring the volume and absorbance of the sample, you can measure precisely the volume of that loop. The volumes to be measured lie in the range of 15 -300 μl.

1. Calibration method using radioactive standard

   This is best done using 32P-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 32P-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. Prime one of the sample lines with buffer as shown in figure 4a.
   ii. Load the other sample line with radioactive solution such that you fill the sample line, the reaction loop, and the exit line entirely and excess solution comes out of the exit line. You now have radioactive sample completely loading the lines as shown in Figure-4b.
   iii. By pushing buffer from the quench load syringe 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 left then with radioactivity distributed as shown in Figure-4c.
   iv. By flushing solution from the third reactant syringe load port (“C”), you will force out the contents of the reaction loop. Counting that sample will provide a measurement of the volume contained within the reaction loop. You are left with radioactivity distributed as shown in Figure-4d
   v. By flushing with a solution from the first reactant syringe load port (on the side not used to load the radioactive sample), you then expel the contents of the “T” loop into a scintillation vial. This sample provides a measurement of the volume contained within that “T” loop. Now radioactive solution is contained only in the sample loop which was chosen for loading (Figure-4e).
   vi. By flushing with buffer from the second buffer syringe load port (the side used for loading the radioactive sample), 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 loops, 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:

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

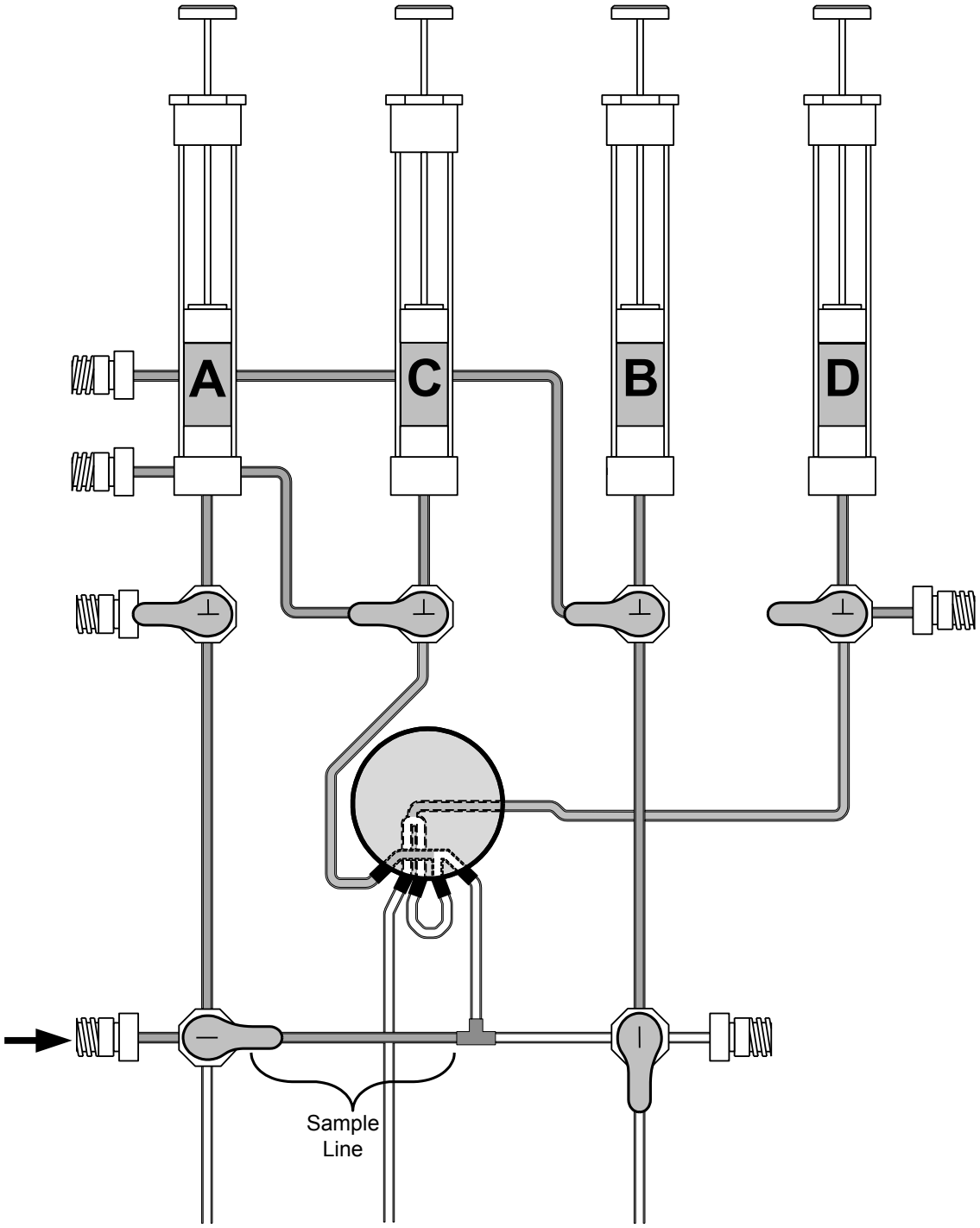
2. Calibration method using an absorbance standard

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. Prime one of the sample lines with buffer as shown in figure 4a.
ii. 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 4b.
iii. By pushing buffer from the quench load syringe 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 is Figure 4c.
iv. By flushing with a solution from the first reactant syringe (one not used to load the absorbance standard sample), you then expel the contents of the reaction loop into a test tube. Measure the volume and absorbance of this sample, which will provide a measurement of the volume contained within that reaction loop. Now absorbance solution is contained only in the sample loop which was chosen for loading (Figure 4d).
v. By flushing with buffer from the second buffer syringe load port (the side used for loading the absorbance standard), 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 loops, 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.

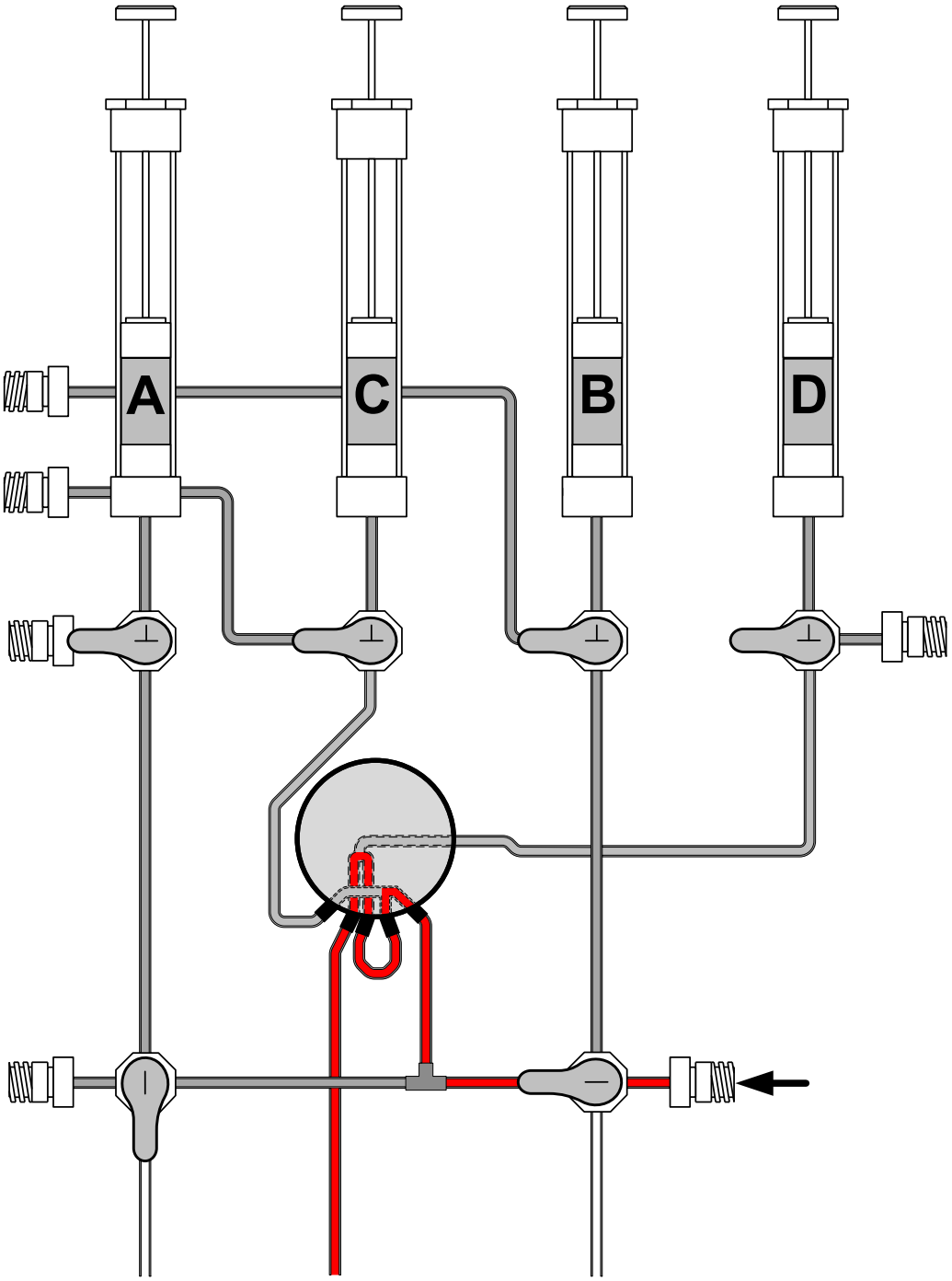
Compute the volume of each internal loop according to the formula:

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



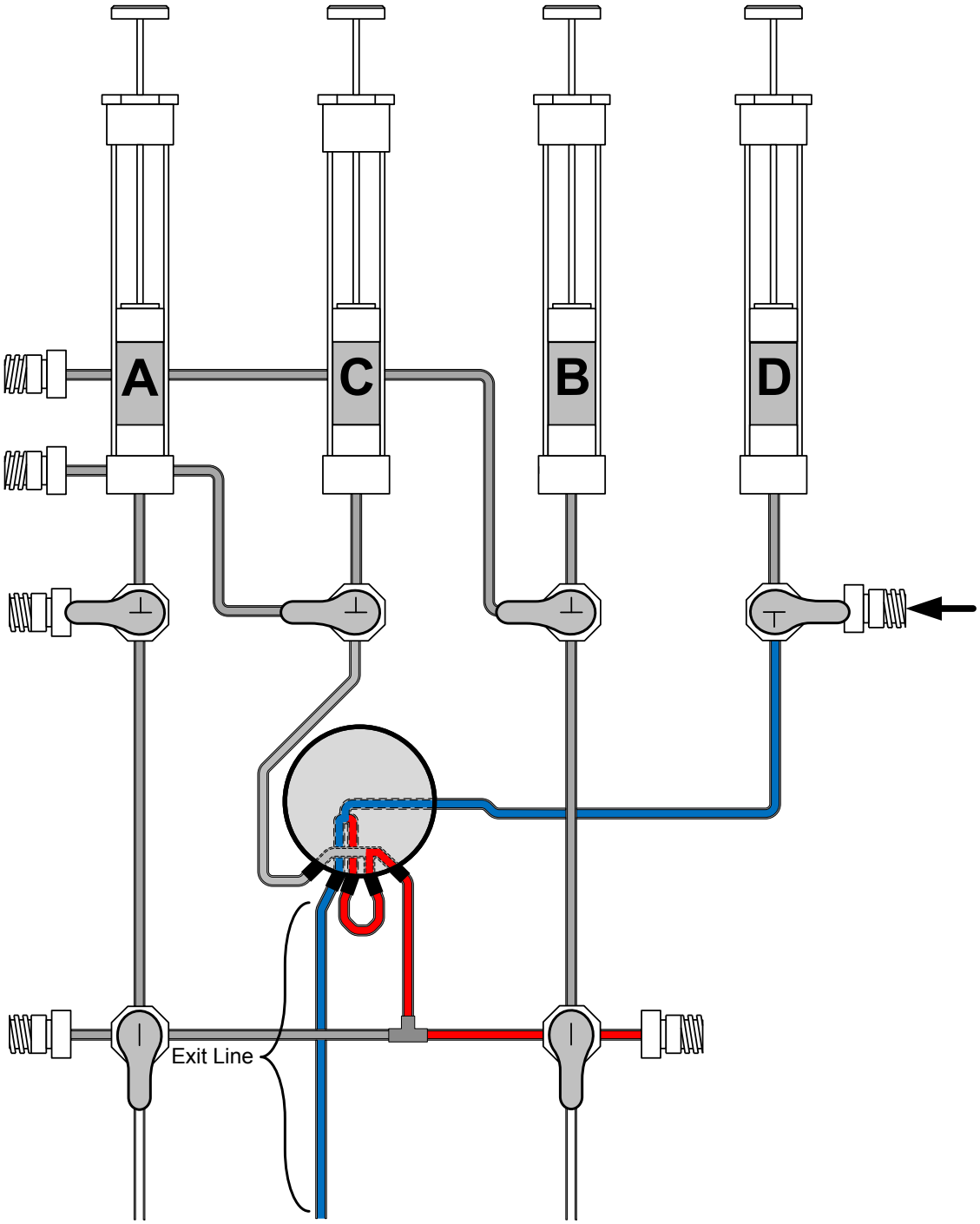
4a. Prime Sample Line

Figure-4a: Prime Sample Line. With the valves in positions as shown, fill the sample line up to the "T" meniscus as shown here. The Grey line indicate they are primed with buffer.



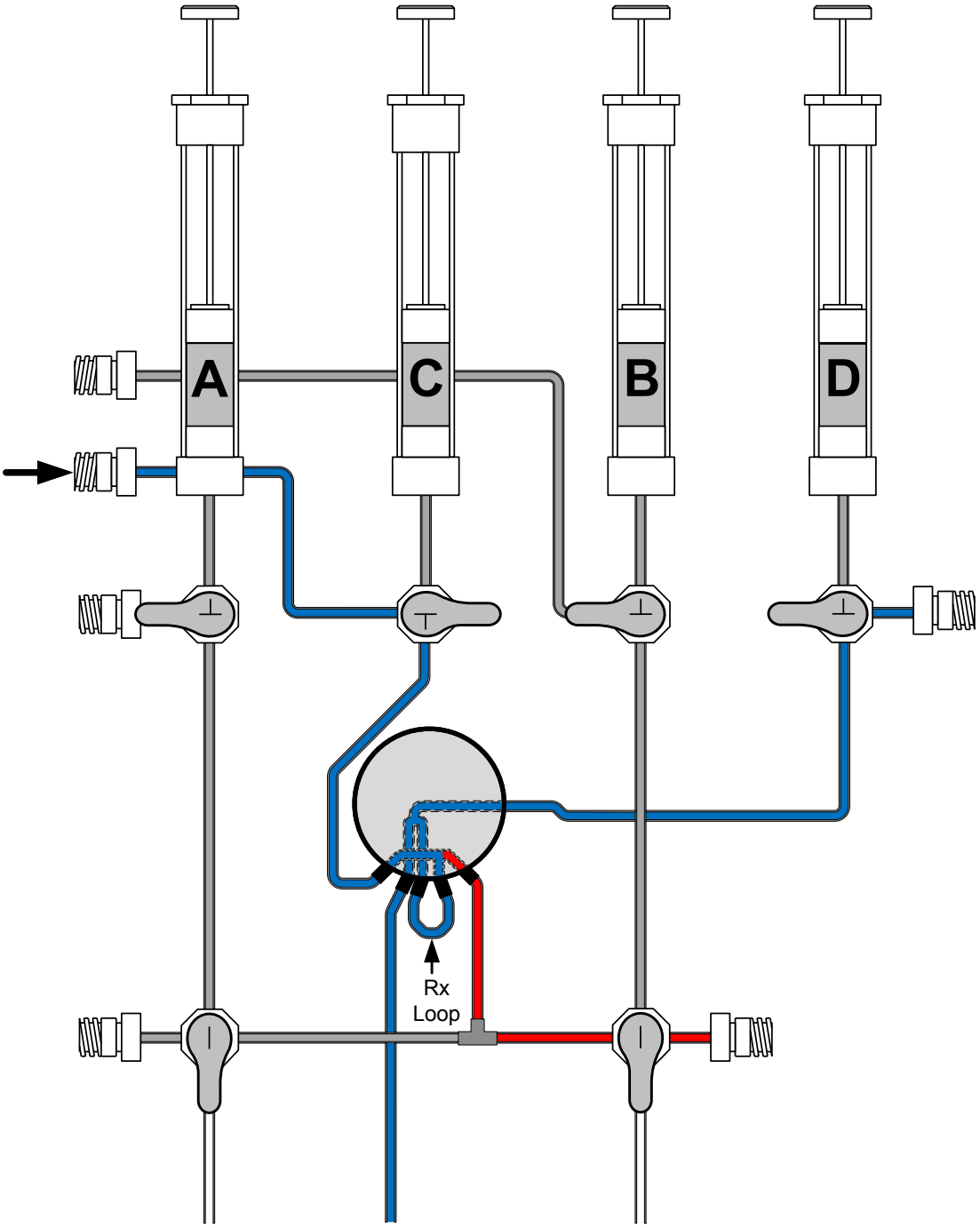
4b. Load Label

Figure-4b: Load Label. With the valves in positions as shown, fill the sample line, the "T" Loop, Reaction Loop and Exit Line with radioactive label as shown here. The red lines indicate that they are loaded with label.



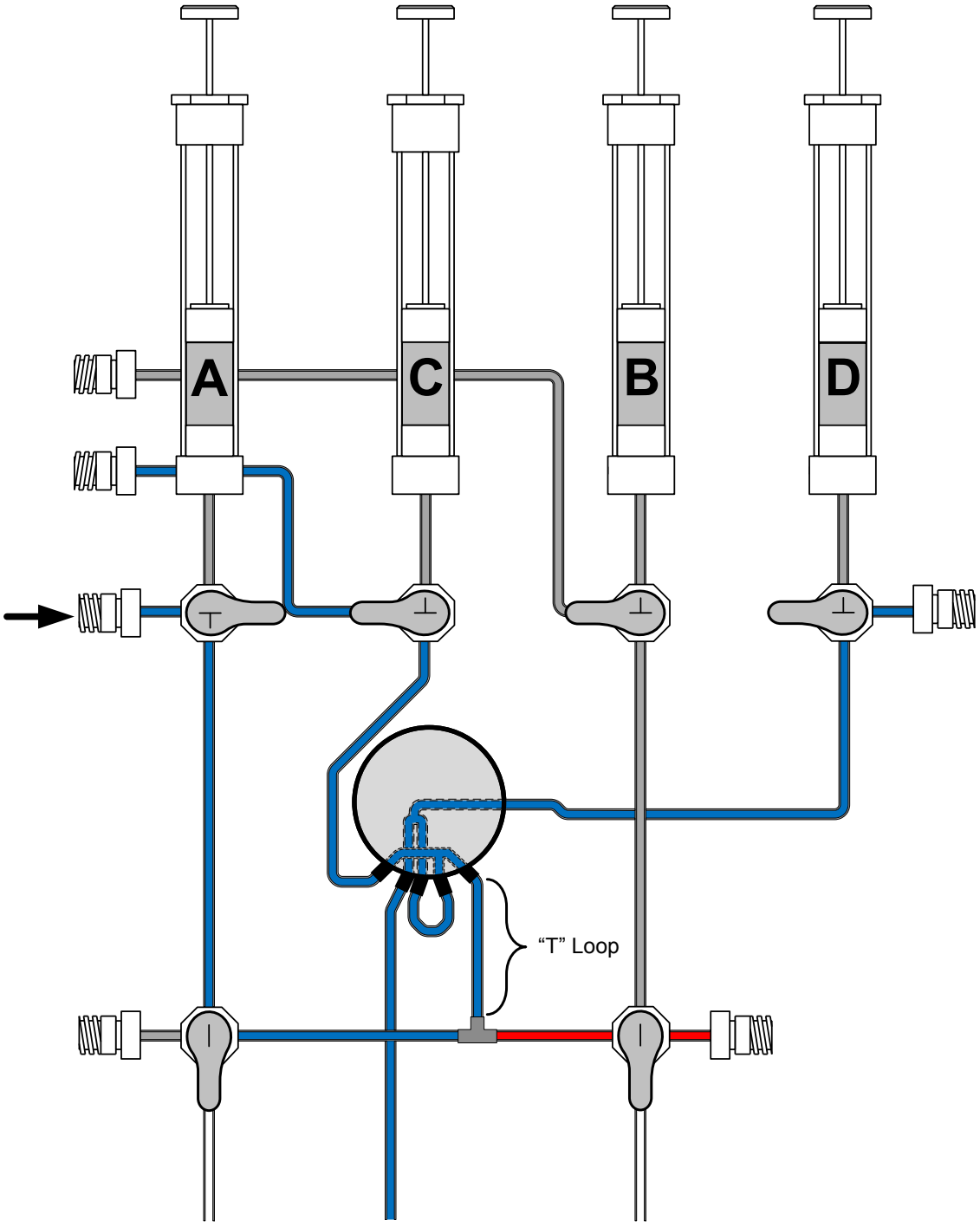
4c. Exit Line

Figure-4c: Exit Line. With the valves in positions as shown, push buffer through the system to completely expel the label in the exit line from the system.



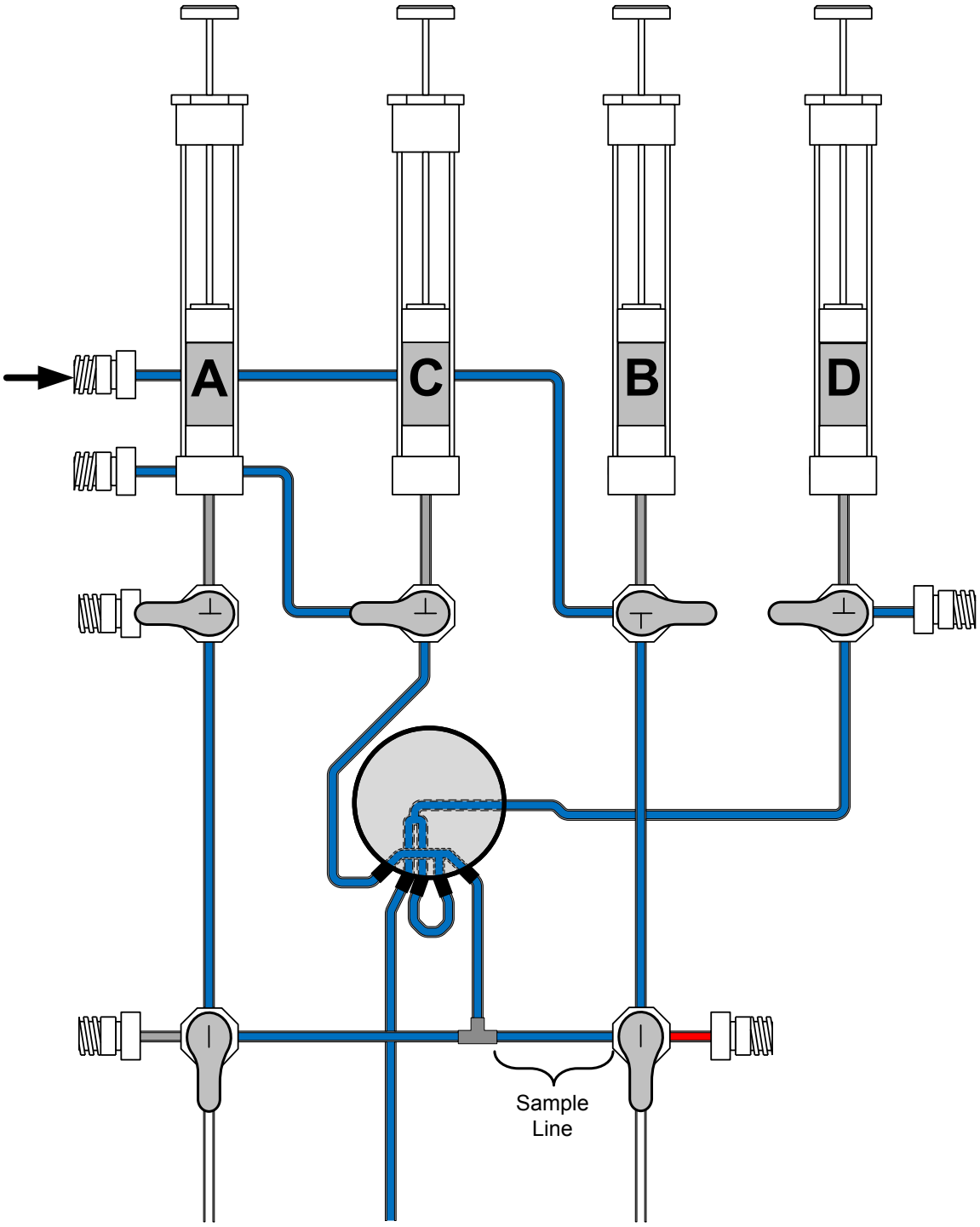
4d. Reaction Loop

Figure-4d: Reaction Loop. With the valves in positions as shown, push buffer through the system to completely expel the label in the reaction loop from the system.



4e. "T" Loop

Figure-4e: "T" Loop. With the valves in positions as shown, push buffer through the system to completely expel the label in the "T" loop from the system.



4e. Sample Line

Figure-4e: Sample Line. With the valves in positions as shown, push buffer through the system to completely expel the label in the sample line from the system.

C. Sample Loop Volumes

To determine the volume delivered by each of the sample loops, load each sample loop 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 flush with buffer from the syringe loading port, collecting the contents into a scintillation vial and counting or into a test tube and weighing and measuring the absorbance if an absorbance standard is used. 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.

D. 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 water in a test tube which has been pre-weighed. This is accomplished using Option 6 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 which is delivered. Using the 5 ml syringes, this should give about 846 μl per revolution for 2 syringes.

E. 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.

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

The calibration 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 = (50.9 μl)*60 sec/min / (846 μl/rev * 220 rev/min) = 16.1 msec

Because we use 220 rpm as the minimum run speed, this calculation shows that 16.1 msec is the longest reaction time that can be obtained using Loop 3. To get a 15 msec time point using loop 3:

Run Speed = (50.9 μl)*60 sec/min / (846 μl/rev * 0.015 sec) = 240 rpm

When operating in the delay mode to determine 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	1820	5.2
2	3	35	1980	11.3
3	8	51	2150	16.3
4	15	85	2480	27.5
5	23	134	2880	43.1
6	31	170	3300	54.7
7	40	200	3620	64.2
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 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 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 SET SPEED & DISTANCE routine (option 3) as outlined below.

First, calculate the motor speed in rpm to obtain a 10 msec reaction time. You should maintain a motor speed in excess of 150 rpm, so a sample loop should be selected to obtain a sufficient speed. For a 10 msec time point, loop 2 would be used (see Table I) which has a reaction loop volume of 35.2 μl for the value described in the table. We also use the volume delivered from the syringes per revolution of the motor, measured as described under CALIBRATION OF SYRINGES. A standard value is 846 μl per revolution. These volumes may vary, so calculations should be based upon the calibration of your instrument. The motor speed is calculated according to the following formula:

Speed = loop volume (μl)/ reaction time (sec)*revolution/ 846 μl*60 sec/1 min

Example: Speed = 35.2 μl/ 0.01 sec * 1 rev/ 846 μl * 60 sec/min = 250 rpm

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 keypad 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 perhaps in the short run would be to apply a vacuum to the exit line 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 the <GO> button. 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 the 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 torque wrench provided in the tool kit, available from KinTek that can be used to get the right torque on the screws. 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.kintekcorp.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.

Dyna-Pump Model 3:

Fisher Scientific Cat. No. 01-092-10
VWR Cat. No. 54904-050

Temperature Probe: A temperature probe can be installed by replacing the plug in the right side of the plastic box with the compression fitting listed below to use in conjunction with the platinum resistance probe. Alternatively, the YSI thermistor probes, type 410 or type 416 (Thomas Scientific Cat. No. 9339-R30 or 9339-R35) can be used.

Omega Engineering, Inc
P.O. Box 2669
Stamford, CT 06906
Phone No: [800]-826-6342
FAX: [203] 359-7700

Quantity	Part number	Name
1 ea	PR-11-2-100-1/8-6-E	RTD probe
1 ea	SSLK-18-18	Compression Fitting
1 ea	MDSS116-MC2	Benchtop Digital Thermometer

Circulating Water Bath: A circulating water bath for temperature control may be connected at the two side ports of the RQF-3 chamber. We recommend a Neslab RT-111 water bath available directly from Neslab Incorporated or from VWR Scientific.

PO Box 1178
Portsmouth, NH 03802
800/4NESLAB
603/436-9444

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 Valves:
2 @ 5 cm
1 @ 7 cm

Sample Load Valves:
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/Optical Flow Cell Chamber for RQF-3 and the Complete RPL-3 System
27-150	Sample Load Loops, 15μl (not shown)
27-155	Circulating Water Fitting
27-161	3-way Upper Drive Syringe Loading Valve
27-162	3-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	Leur Lok Syringe Connecting Adapters
27-205	Side Panel Mounts (screw into plexi-glass box)
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
27-333	Additional/Replacement Optical Flow Cell for the RPL-3 Chamber
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.

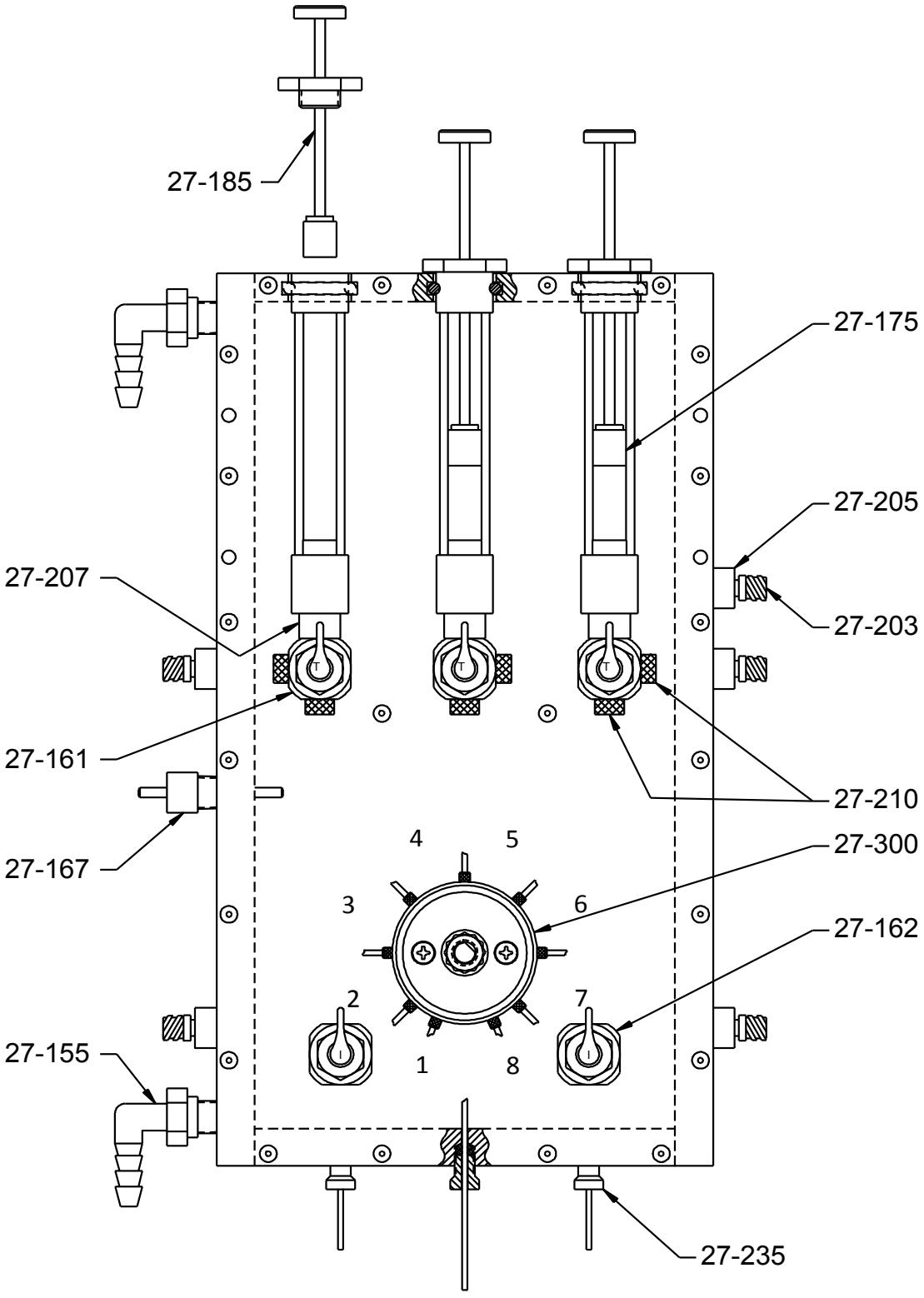


Figure 4. ***Part Designation of Quench-Flow System.*** **The part numbers are shown in this figure to assist with the ordering of replacement parts or systems. Please call for pricing.**