KinTek RQF-3 Quench Flow Instrument
Kintek Corporation
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Rapid-quench kinetic analysis has become the method of choice for examination of enzyme reaction pathways. The KinTek RQF-3 Quench-Flow Instrument enables the most definitive experiments to be performed most efficiently, preserving precious biological samples and providing accurate, reliable data.

The Chemical Quench-Flow Method

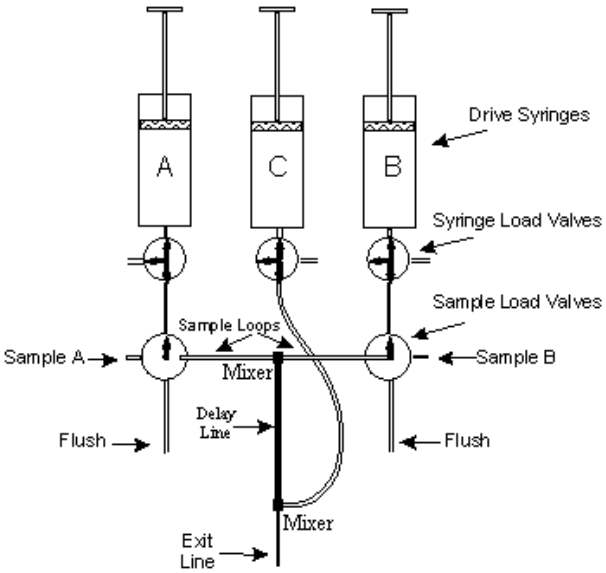
Stopped-flow techniques are limited by the fact that one does not always have an optical signal for the reaction of interest and the optical signals cannot be interpreted rigorously if the extinction coefficients of intermediates or products are not known. In these cases direct measurement of the conversion of substrate to product is required. Chemical quench-flow methods allow such a measurement.

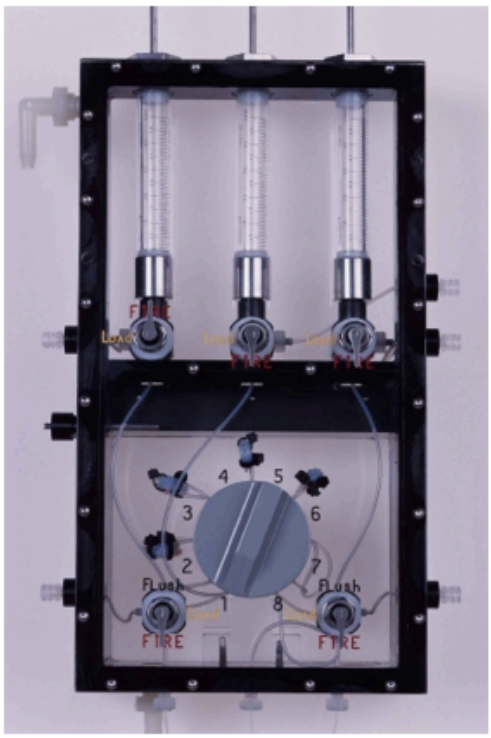
A basic chemical quench-flow allows the mixing of two reactants, followed (after a specified time interval) by quenching with a chemical agent (usually acid or base). A drive motor is used to force reactants contained in drive syringes together into a mixer after which the mixed reactants pass into a reaction delay line. After passing through this delay line the reaction passes through another mixer where chemical quench is added to terminate the reaction. The quenched sample is then collected and analyzed to quantitate the conversion of product to substrate (usually the substrate is radiolabeled and product formation is determined by either gel electrophoresis or standard chromatography methods). The duration of the reaction is determined by the volume of the reaction delay line and the flow rate of the reaction through the delay line. In practice, the reaction time is varied by changing the length of tubing in the reaction loop and to a certain extent the flow rate through the delay line. The apparatus is then flushed and a new reaction delay line is selected to obtain a different reaction time. By selecting various delay lines reaction time of 2 to 100 milliseconds can be obtained. This simple design limits the maximum attainable reaction time by the maximum delay line size. Longer reaction times cannot be obtained by using slower flow rates because it is necessary to use rapid rates of flow to maintain turbulent flow necessary for efficient mixing. A 40 cm reaction loop (which contains 200 microliters) can provide a 100 millisecond reaction time (very long delay lines are unwieldy). This design also wastes precious reactants, by using the reactants to push themselves though the delay line. This means that after each time point the delay line is full of reactants. Therefore after the delay line is changed, it contains unused reactants (as much as 200 microliters at a time), which must be discarded.

To minimize sample volumes without any wasted reactants between different reaction times and to easily achieve reaction times longer than 100 milliseconds, KinTek Corporation designed the RQF-3 Rapid Chemical Quench-Flow. In our RQF-3 Rapid Chemical Quench-Flow the two reactants are loaded into small loops of tubing containing 15 microliters solution. A three-way valve is then used to put the loaded sample loop in line with the drive syringe containing the buffer. The drive syringes are then used to force the reactants together through the delay line to the point of mixing with the quenching solution, followed by sample collection. The delay line size in the RQF-3 is controlled by our innovative eight-way valve, which allows the user to put the correct loop in position by the simple twist of a handle. Using the delay lines provided on the eight-way valve reaction times from 2 milliseconds to approximately 100 milliseconds. For longer reaction times (from 100 milliseconds to infinity) the RQF-3 utilizes the computer-controlled stepped motor to operate in push-pause-push mode. The first push is used to mix the reactants in the delay line. There the motor pauses, allowing the reaction to continue, and then the pause is followed by a second push which mixes the reactants with the quench solution. In this fashion, the RQF-3 makes it simple to perform quench-flow experiments for any time course the user desires.

With our computer-controlled servo motor design the RQF-3 can perform sophisticated double mixing experiments. In this type of mixing experiment the first mixing occurs as described above, however when the mixed reactants exit the delay line into the exit line they are mixed with a third reactant rather than a quench solution. The computer then pauses the stepping motor, which holds the reactants in the exit line for a time entered by the user. The computer then fires the motor again and the reactants are pushed into a collection tube containing a chemical quench. This methodology has been used many times to perform experiments that require double mixing, such as pulse-chase experiments.

RQF-3 Valve System





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