

MODEL SF-2004 STOPPED-FLOW

The KinTek SF-2004 Stopped-Flow meets the most demanding standards for a research instrument. The instrument's unique design is based upon 25 years of research experience in stopped-flow kinetics, and has been computer-designed starting at the heart of the instrument; the drive system and the observation cell. The KinTek Stopped-Flow utilizes a new servomotor-drive system for unparalleled performance, giving the operator precise control over drive rates and volumes with the utmost reproducibility. The programmed motor control provides the necessary instantaneous stop of

solution flow. It also allows double mixing experiments to be performed with exceptional ease and precision. The observation chamber and drive syringes were designed for analysis of small volumes of precious biological samples. Less than 20 microliters of each sample is required for each reaction and less than 50 microliters is wasted in flushing and loading each syringe with a new reactant. The light source and optical path have been optimized to provide the best signals for either fluorescence or absorbance measurements. It is the best stopped-flow ever built...and it keeps getting better!

- ⇒ **Absorbance/Fluorescence T-cell Configuration**
- ⇒ **Computer-controlled Vertical Syringe Drive**
- ⇒ **PC Computer with Windows® Software**
- ⇒ **Single- or Double-Mixing Experiments**
- ⇒ **Interchangeable Sample Cells**
- ⇒ **Full Line of Accessories**



COMPUTER CONTROL

The KinTek Stopped-Flow is entirely under computer control from instrument setup to data collection and analysis. The PC-compatible computer with a Pentium IV processor and 400 kHz A/D-D/A converter provide fast and accurate data collection, analysis and instrument control. The operation of the instrument is easy and intuitive so the operator is free to concentrate on the data collection and analysis. The computer controls the instrument drive system, the detector gain, and checks to

see that the sample load/fire valve is in the right position. It is also programmed to assist in a simple calibration of the instrument. The instrument and software have been integrated into a system that is a pleasure to use. Our use of a PC computer provides compatibility and access to a wealth of software for specialized data analysis and graphics presentation. The computer can easily be networked to allow data to be shared with other members of the lab or colleagues around the world.

NEW! SERVO MOTOR DRIVE

The powerful computer-controlled servo motor drive and high helix drive screw provide fast drive rates with instantaneous starts and stops. We provide a fast servo motor with programmable acceleration and deceleration for unparalleled performance. Acceleration to full speed is smooth and accurate, while the fast deceleration insures a nearly instantaneous stop to achieve the shortest dead times. You will be amazed by the smooth power of the New SF-2004.

UNIQUE CAPABILITIES OF THE MOTOR DRIVE

In addition to providing new standards for reliability and performance, the stepping motor drive system allows new advances in the way experiments can be performed. For example, it allows automated collection of multiple, replicate reactions to increase signal-to-noise. Moreover, double-mixing experiments with three reactants can be performed with ease using computer-programmed reaction delays as described below. In addition, a computer read-out of the motor position provides a calculation of the number of shots remaining in the drive syringes. The systems helps to eliminate operator error and the guesswork in performing an experiment. Only the KinTek Stopped-Flow allows for such optimal performance and versatility.

NEW ACCESSORIES AVAILABLE

The precise servo-motor drive systems enables two important accessories to be used with the stopped-flow. A Titration Module enables accurate equilibrium titrations to be performed with a sample continuously stirred while small volumes of titrant are added. In addition, a Quench-Flow Accessory enables simple chemical quench-flow experiments to be performed using the stopped-flow motor drive. These critically important accessories allow for a more rigorous interpretation of stopped-flow data.

VERTICALLY MOUNTED DRIVE CHAMBER

The KinTek Stopped-Flow uses a solid vertical drive mount for ease of use and to aid in the expulsion of air bubbles. This arrangement places the sample syringes in a position where they are easy to load and to observe through the transparent water jacket. Various sample chambers can be easily interchanged or removed for cleaning. The reaction block and sample chamber are thermostatted by use of a circulating water bath (provided separately).

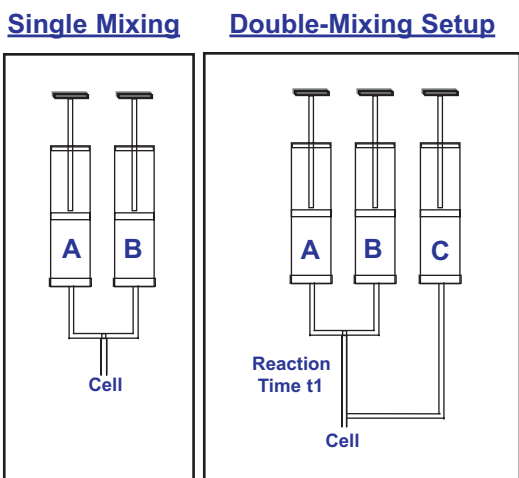


TEMPERATURE CONTROL

Water circulates on all six sides of the observation cell held in the center of the reaction block. The drive syringes are contained within a sealed plexiglass chamber to allow optimal thermostating and easy observation of the syringe contents. The computer records and displays the temperature using a digital sensor mounted in the sample chamber. The samples contact only glass, teflon and Kel-F. Thus there are no potential problems from metal contamination or the use of organic solvents, acids or bases.

DOUBLE MIXING EXPERIMENTS

The KinTek Stopped-Flow comes with three drive syringes. In the normal mode of operation, only two syringes are connected and used to drive the mixing of two reactants (A and B) before they flow into the observation cell. Alternatively, the third syringe can be connected to perform a double mixing experiment. Two reactants are mixed and then flow into a delay line allowing the first reaction to occur. The motor then stops for the desired reaction time and then on the next push, the solution is mixed with the third reactant (C) and forced into the observation cell. The reaction time is under precise computer control, allowing any ageing times from 5 msec to many minutes. No other instrument offers such short ageing times with such small sample volumes.



SMALL SAMPLE VOLUMES

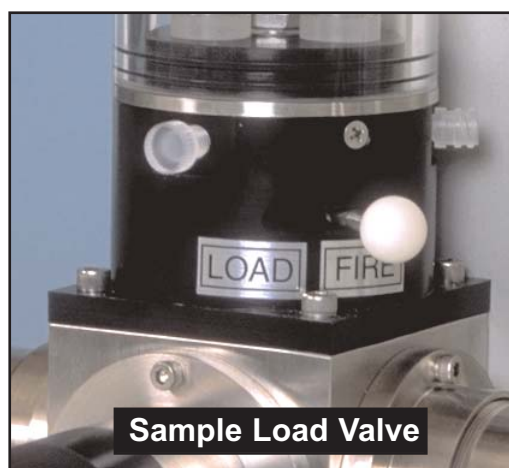
The KinTek Stopped-Flow is designed to conserve precious samples. Each shot requires only 15-20 microliters of each reactant. Moreover, by minimizing the volume of the sample lines connecting the loading ports to the sample syringes, the instrument requires only a small load volume. Typically, 6-8 replicates can be obtained from only 250-300 microliters of each reactant.

SIMULTANEOUS FLUORESCENCE / ABSORBANCE

The KinTek Stopped-Flow has been designed specifically to meet the more stringent requirements of fluorescence measurements requiring an intense, stable light source and a sensitive detection system, providing the best fluorescence sensitivity and optimal absorbance measurements. Absorbance and two channels of fluorescence can be measured simultaneously. Optional polarizers allow simultaneous measurement of both perpendicular and parallel polarized light for real time anisotropy.

SAMPLE LOAD VALVE

A unique two-position valve is used to allow easy and efficient sample loading. In one position, the three syringes are connected to their individual sample load ports. In the other position, the three syringes can be driven to force the mixing of reactants into the observation cell. Optical sensors read the position of the sample load valve and tell the computer whether the valve is in the LOAD or FIRE position. The computer will not fire a shot if the valve is in the wrong position. This safety feature further conserves precious samples. The sample load valve is designed based upon rotating disk technology to give years of trouble-free service.



CHOICE OF OBSERVATION CELLS

The observation cell and sample chamber can be purchased as a complete unit, allowing easy and rapid interchange between sample cells. Three observation cells are available, each with its unique capabilities to optimize data according to the demands of your experiment. Other cells can be custom manufactured to your specifications.

Fluorescence Observation Cell: A 5 mm pathlength and a 1 x 2 x 5 mm window for observation provide the optimum geometry for fluorescence data collection and absorbance measurements. *This is the standard cell provided with the instrument.*

Long Pathlength Observation Cell: This cell has a 25 mm pathlength to provide optimal sensitivity for absorbance measurements while still allowing a good fluorescence signal.

Short Pathlength Observation Cell: A 2 x 2 mm square observation cell is recommended for solutions having a high absorbance. This cell give optimal fluorescence or absorbance measurements for solution with a high absorbance of the incident light without sacrificing signal.

STOPPED-FLOW SYSTEM PERFORMANCE

There are three variables that establish instrument performance: The dead time is a measure of the time it takes for samples to flow from the point of mixing to the point of observation; mixing efficiency is a function of how completely the solutions are mixed prior to entering the observation cell; and signal-to-noise ratio is a function of the optical system performance and data collection methods. The KinTek Stopped-Flow achieves superior performance by optimizing each aspect of data collection.

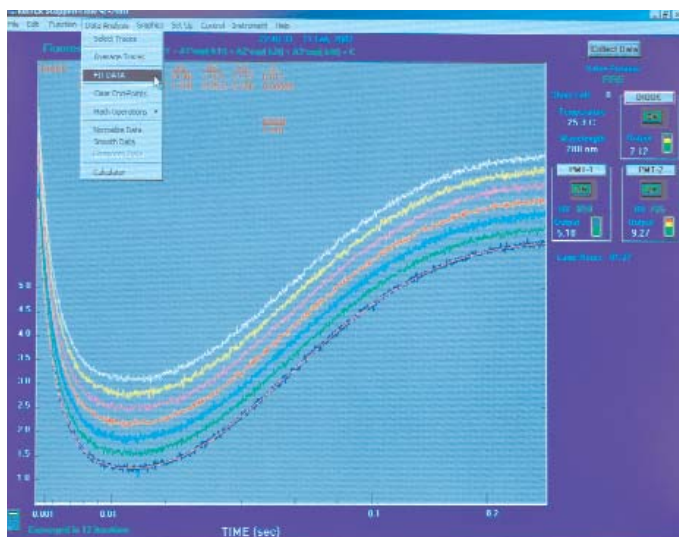
Short Dead Time. Signal-to-noise ratio and dead time dictate the whether an instrument can measure a given reaction. The standard for comparison of stopped-flow instruments is the dead time calculated by measurement of the reaction amplitude and rate of a well-defined reaction. The KinTek Stopped-Flow has a measured dead time of 0.9 msec. The motor drive system provides a reproducible and reliable dead time that you can count on.

Mixing Efficiency. The KinTek Stopped-Flow uses Berger Ball mixers or KinTek's proprietary Jet-Mix mixer. Each provides efficient mixing with minimal back pressures. Efficiency of mixing is not a matter for theoretical debate. Test reactions have proven that mixing is completed in the KinTek Stopped-Flow before the sample enters the observation cell.

Optimal Signal-to-Noise Ratio. The signal-to-noise ratio ultimately limits the quality of the data, the useful application of the dead time and the confidence in the conclusions that can be obtained. The KinTek Stopped-Flow provides several unique advances that optimize the signal-to-noise ratio. A high intensity, *Super-Quiet* arc lamp source is used to illuminate the sample. The light source uses a parabolic mirror and lens-free optics to provide optimal throughput to a monochromator. Light from the monochromator is fed through a quartz optical fiber to the sample chamber, simplifying the optical alignment and providing efficient, uniform illumination of the sample. The optical path for light detection is free of lenses which would limit light throughput. Especially for fluorescence detection, the light path provides the most efficient recovery of emitted light and therefore the highest signal-to-noise. Finally, the electronic circuits have been computer designed to provide the most noise-free amplification possible and the data collection uses state-of-the-art methods to improve signal-to-noise ratio as the data enters the computer in digital form. The entire system is integrated to provide the best possible signals in any given situation.

SOFTWARE

Computer programs for data collection and analysis run on an IBM compatible computer under Window XP®. A highly oversampled A/D converter provides optimal signal/noise, which can be further improved by numerical filtering. Data can be collected on one, two, or three channels simultaneously and on a linear or logarithmic time scale. Data can be fit by nonlinear regression to either one, two, or three exponentials or to a burst equation with one or two exponentials preceding a linear phase. Multiple traces can be averaged to further improve signal-to-noise. Data and the computer-generated fits are displayed on the video terminal and can be printed to any printer supported by Windows®. The user interface is intuitive, taking advantage of modern event-driven programming and pull-down menus to make the software easy to learn and yet powerful in its capabilities.



FREE SOFTWARE UPGRADES!

KinTek offers free software upgrades for the lifetime of the instrument. Unlike other companies that charge extraordinary fees to upgrade the software, we protect your investment in a KinTek stopped-flow by allowing you to benefit from the advances in software that occur as we continue to improve our instruments.

HARDWARE UPGRADES

KinTek is continually improving its instruments. To protect your investment, we offer reasonably priced upgrades of you system to take advantage of the new advances. Your instrument will not become obsolete in just a few short years!

DEAD TIME DETERMINATION

Several methods are available to measure the dead time in a stopped-flow. The dead time for the SF-2002 was determined by following the quenching of N-acetyl-tryptophanamide (N-AcTrpNH₂) fluorescence by N-bromo-succinamide (NBS). The rate of the reaction varies linearly with NBS concentration, and the variation of the observed amplitude of the reaction with rate of reaction defines the dead time of the instrument. Figure 1 shows the fluorescent traces upon reaction of 5 μM N-AcTrpNH₂ with various concentration of NBS. The data were fitted to a single exponential:

$$F = F_0 e^{-kt} + C \quad (1)$$

where F represents the fluorescence intensity at time, t , F_0 is the calculated initial fluorescence at the time of mixing, and k is the rate constant. The fitted curves all extrapolate to an intersection at the starting time of the reaction.

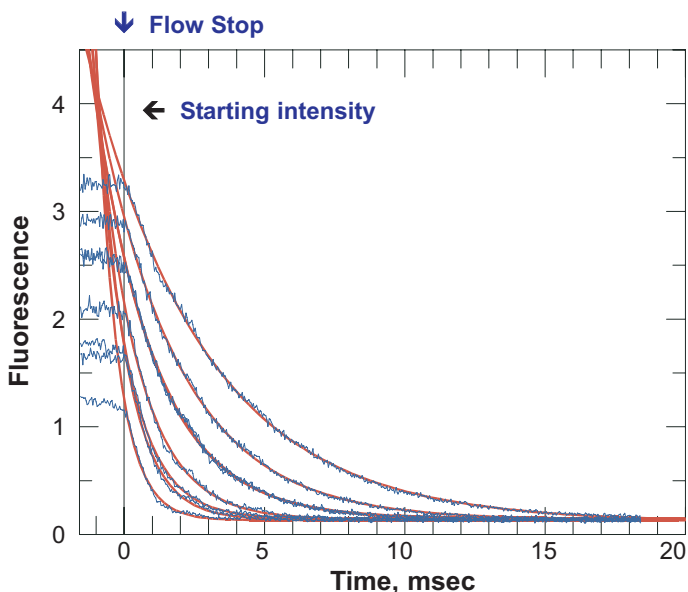


Figure 1. Time course of fluorescence quenching. Fluorescence was excited at 280 nm and observed through a 340 nm band pass filter. Zero time in this graph is defined by the instant that flow stops. Traces are shown for concentrations of NBS of 312, 500, 625, 750, 1500 and 2000 μM according to the method of Peterman (Analytical Biochemistry 93, 442-444, 1979). The red curves were obtained by fitting to a single exponential by nonlinear regression using KinTek software.

In Figure 2A, we plot the rate of reaction as a function of NBS concentration. This graph shows that the rate of the reaction increases linearly as a function NBS concentration. The slope defines a second order rate constant of approximately $7 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$. Note also that the fastest rate measured was 1600 s^{-1} . Given the signal:noise ratio seen in Fig. 1, higher rates could have been measured at higher concentrations of NBS.

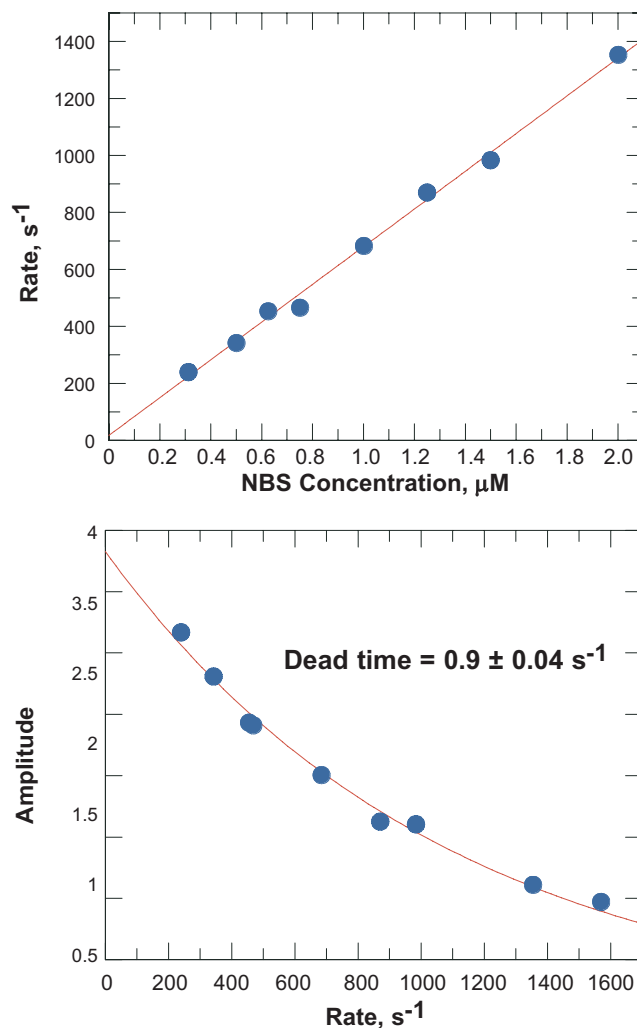


Figure 2. Concentration dependence of the rate and amplitude. **A.** Rates obtained by fitting curves in Fig. A are plotted versus NBS concentration. **B.** Amplitudes are plotted as a function of the rate obtained by fitting curves in Fig. A. The line shows the fit to equation (2) by nonlinear regression.

Figure 2A shows a plot of the observed amplitude of the reaction as a function of the measured rate constant from the fits seen in Figure 1. The amplitude observed after flow stops, F_{obs} , can be fit to the equation:

$$F_{\text{obs}} = F_0 e^{-kt} + C \quad (2)$$

When data were fitted to this equation by nonlinear regression, the dead time was calculated to be $0.9 \text{ ms} \pm 0.04 \text{ ms}$ as shown by the smooth curve. These data define the dead time of the instrument to be 0.9 msec and demonstrate the quality of the data that can be expected from the KinTek SF-2002.

FLUORESCENCE ANISOTROPY

When a fluorophore is excited by polarized light its emitted light is also polarized. The emitted light can be depolarized by several phenomena including rotational diffusion of the fluorophore. The extent of depolarization of the emitted light reveals the average angular displacement of the fluorophore between the time of excitation to the time of emission. The rate of rotational diffusion by the fluorophore can be changed by the binding of a second molecule. Thus by monitoring the change of emitted polarized light from a fluorophore, the kinetics of binding can be directly measured.

The change in polarization by the emitted light can be expressed terms of polarization (P) or anisotropy (r) where I represents the intensity of the light emitted perpendicular or parallel to the incident light.

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \quad r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

Figure 3 shows a single trace following the binding of 0.1 mM Phloxine B to 1 mM BSA. The KinTek Stopped Flow simultaneously recorded the intensity of light emitted through polarizers perpendicular and parallel to the exciting light. It then calculated and displayed the anisotropy. As shown in the figure, the anisotropy increases as a single exponential with a rate of 0.06 s⁻¹ providing a direct measurement of the rate of binding.

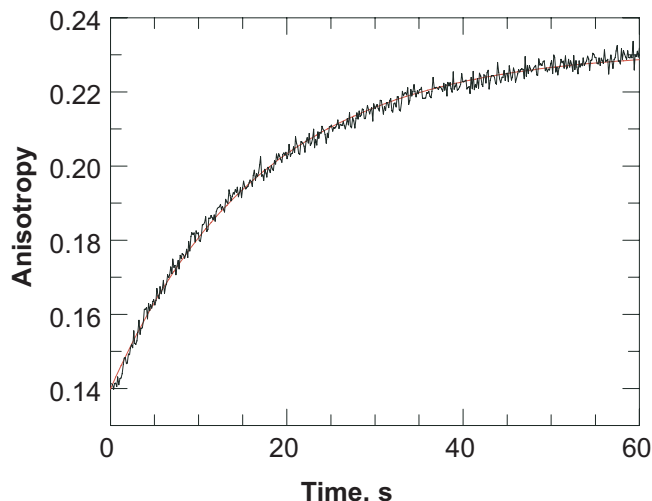


Figure 3. Fluorescence anisotropy. 0.1 mM Phloxine B was mixed with 1 mM BSA, in 50 mM phosphate buffer at pH 7.4. Fluorescence was excited at 500 nm with plane polarized light and emission was recorded through 550 nm cutoff filters. The intensity of light emitted perpendicular and parallel to the incident plane polarized light were measured simultaneously and anisotropy, r, was calculated. The red curve shows the fit to a single exponential.

The Fluorescence Anisotropy Accessory includes a set of three polarizers to allow the simultaneous measurement of light emitted perpendicular and parallel to the incident light. An second photomultiplier tube and high voltage power supply are also needed.

CCD ARRAY AND SPECTRAL ANALYSIS SOFTWARE

The KinTek SF-2004 can be equipped to record spectra in real time in the stopped-flow by the use of a CCD array detector. The CCD (charge-coupled device) array allows simultaneous measurement of 2048 different wavelengths, ranging from 240 nm to 750 nm, every 1.5 milliseconds. This is the highest resolution in time and wavelength of any stopped-flow system! Moreover, the CCD array has better sensitivity for measurement of low levels of light than the standard diode array. The CCD array integrates signal over time so you can increase sensitivity by longer integration times. For example, you can measure changes in fluorescence spectra every 100 msec.

CCD Spectral Array

SVD: Singular Value Decomposition. The arrays of data collected are analyzed by SVD (Singular Value Decomposition) to deconvolute the spectra of the individual reacting species and their kinetics of interconversion.

The CCD Array Accessory includes the spectrometer, optical fiber, light source and software. The light source provides a stable source of white light to illuminate the sample. The optical fiber then brings the light from the sample cell to the miniature spectrometer/CCD array detector. Call for more information.

SPECIFICATIONS

Motor Drive: A computer-controlled servo motor drive provides precise and reproducible mixing of 2 or 3 solutions. The motor is digitally controlled with a fast programmable power supply to provide essentially instantaneous starts and stops of the drive syringes for repeated or sequential mixing.

Syringe chamber and cell assembly: The syringe/cell assembly can be easily removed for maintenance or for changing of cell types. The syringe chamber is transparent and can be thermostatted by circulating water (provided separately).

Valve position sensors: Optical sensors detect the load and fire positions of the sample load valve to prevent incorrect operation so as to preserve precious samples.

Optical fiber: A quartz optical fiber bundle is used to bring light from the exit slit of the monochromator to the sample cell. This affords optimal signal with easy, precise alignment of the optical path.

Detectors: A UV-sensitive photomultiplier and a UV-enhanced are provided to simultaneous measurement of fluorescence and absorbance. A second (optional) photomultiplier can be used to provide an additional fluorescence channel to simultaneous measurements as two wavelengths or for real time polarization anisotropy measurements.

Software: Stopped-Flow system software runs under Windows XP®, providing precise and complete computer control of data collection, analysis and printing. Data can be easily transported to other PC programs for data analysis and presentation. We also a package for single value decomposition of time resolved spectra.

KinTekSim: Software for computer simulation of complex kinetic processes is provided with each instrument. This software affords fitting of data directly to mechanism using nonlinear regression and global analysis to fit an entire data set to a single reaction pathway.

Syringe volume and range: 5 ml standard. Syringes of 0.25, 0.5, 1.0, 2.0 and 10.0 ml are also available for variable ratio mixing.

Temperature range: A range of 0-70°C is maintained by circulating water bath (provided separately).

Dead Time: The dead time is less than 1 milliseconds. Reaction rates greater than 1600 s⁻¹ have been measured.

Sample Volumes: The minimum reaction volume is 20 µl of each reactant per shot plus 100 µl to flush and load a new sample.

Data Collection and Analysis: 200 kHz 12 bit A/D converter provides fast and accurate data collection. Data can be fit to 1, 2 or 3 exponentials or 1, 2 exponentials plus a linear phase. Fit by nonlinear regression using the Marquat Method. Multiple traces can be averaged to improve signal-to-noise.

Double Mixing Experiments: The standard configuration includes three drive syringes to allow double mixing experiments to be performed. The shortest time for the first aging, the time allow for the first two reactants before adding the third, is only 5 msec. The stepper motor drive affords precise and reproducible control of the ageing time.

Observation Cells: Choice of three observation cells is provided. Each can be used for either absorbance or fluorescence. The dead time is less than 1 ms for each cell and less than 40 µl is required per shot (total volume). Cells can be easily interchanged by purchasing a syringe/cell block assembly.

Computer: Computer specifications are continually improving as the industry produces faster computers with more memory at lower prices. The following lists the minimum configuration of the computer provided with the instrument.

PC computer, Windows XP®

512 MB RAM, 80 GB hard drive

A/D Converter: 200 kHz, 12-bit, 8 channels.

Light Source: Either a 100 watt mercury arc lamp, 75 or 150 watt xenon arc lamp, or 150 watt mercury-xenon lamp can be used. The lamp is surrounded by a parabolic mirror to provide optimal collection of light intensity. Arc lamps can be cooled by circulating water (bath not provided). A highly regulated, current-controlled power supply and igniter provide stable light output (<0.1% ripple after warmup).

Monochromator:

Quarter-meter Czerny-Turner, f/4.

Wavelength range: 220-800 nm

Wavelength accuracy: 0.5 nm

Grating: 1200 lines/mm blazed at 300 nm

Slits: continuously adjustable from 0 to 6 mm.

Bandpass: 4 nm per mm of slit width.

Stray light: less than 0.02%

Computer controlled wavelength drive.

Power Requirements: 110 volts, 15 amps.

Dimensions: (width x depth x height) and weight:

Stopped-flow stand: 48 x 46 x 83 cm 21 kg

Motor controller: 61 x 51 x 41 cm 13 kg

Light Source: 61 x 61 x 35 cm 19 kg

Lamp power supply: 28 x 30 x 11 cm. 7.8 kg.

SERVICE CONTRACTS

The KinTek Stopped-Flow has been designed to provide years of trouble-free use. In the event that any repairs are necessary, prompt service will be provided by our factory personnel. The modular design of the instrument makes it easy to send components to the factory for service. We will also be pleased to provide the needed technical support and supplies to allow qualified technicians at your home institution to perform any simple repairs. Our singular focus is to keep your instrument running and collecting data. As scientists ourselves, we know how hard you work to prepare your precious samples and we will work equally hard to see that you have an instrument ready to do the experiments you have planned.

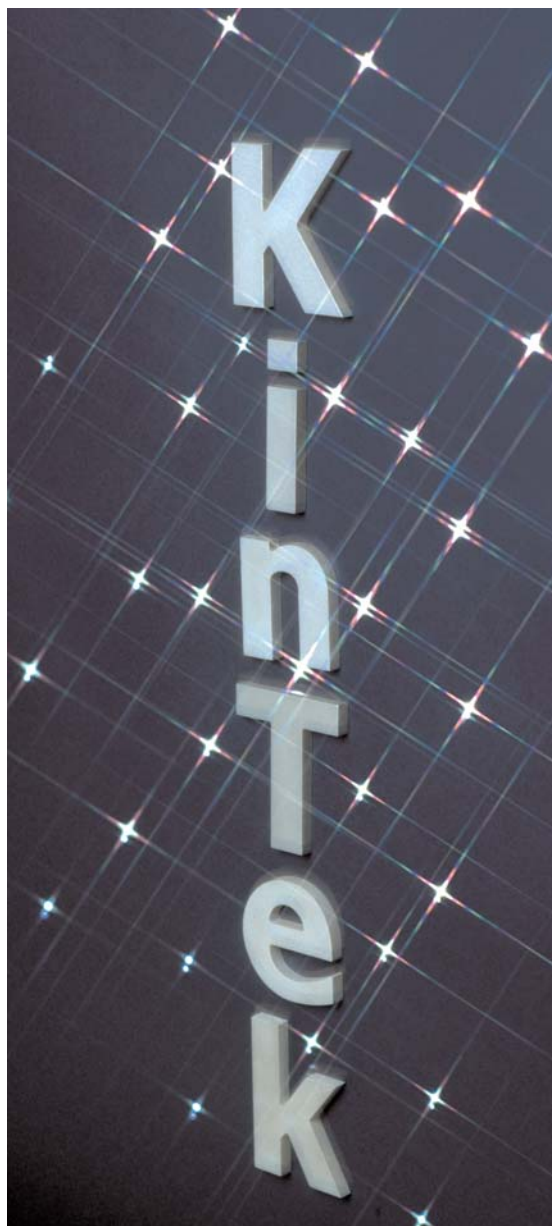
A variety of service contracts are available. Talk to our sales representative to arrange the service contract that best meets your needs.

WARRANTY

The KinTek Stopped-Flow is backed by a 2-year warranty against defects in the motor, power supply/drive unit, and the sample load valve. Only KinTek offers such an extended warranty—because we believe in the reliability of our instruments.

ABOUT KINTEK CORPORATION

KinTek was founded by Kenneth A. Johnson, Ph.D. in 1987. Dr. Johnson needed to design and build new instruments for his own research. The company was founded in response to requests from his colleagues for instruments to do similar experiments. Dr. Johnson's major motivation in starting the company was to encourage others to do the right experiments to directly measure reactions occurring at the active sites of enzymes. The company has continued that tradition, putting the needs of the scientific community first and encouraging innovation. Over 500 instruments have been sold in the past 25 years. KinTek has seen a steady growth in its sales every year since its inception, evidence of the reputation of its instruments within the scientific community.



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