Octet® SF3 SPR

Powered and Prepared

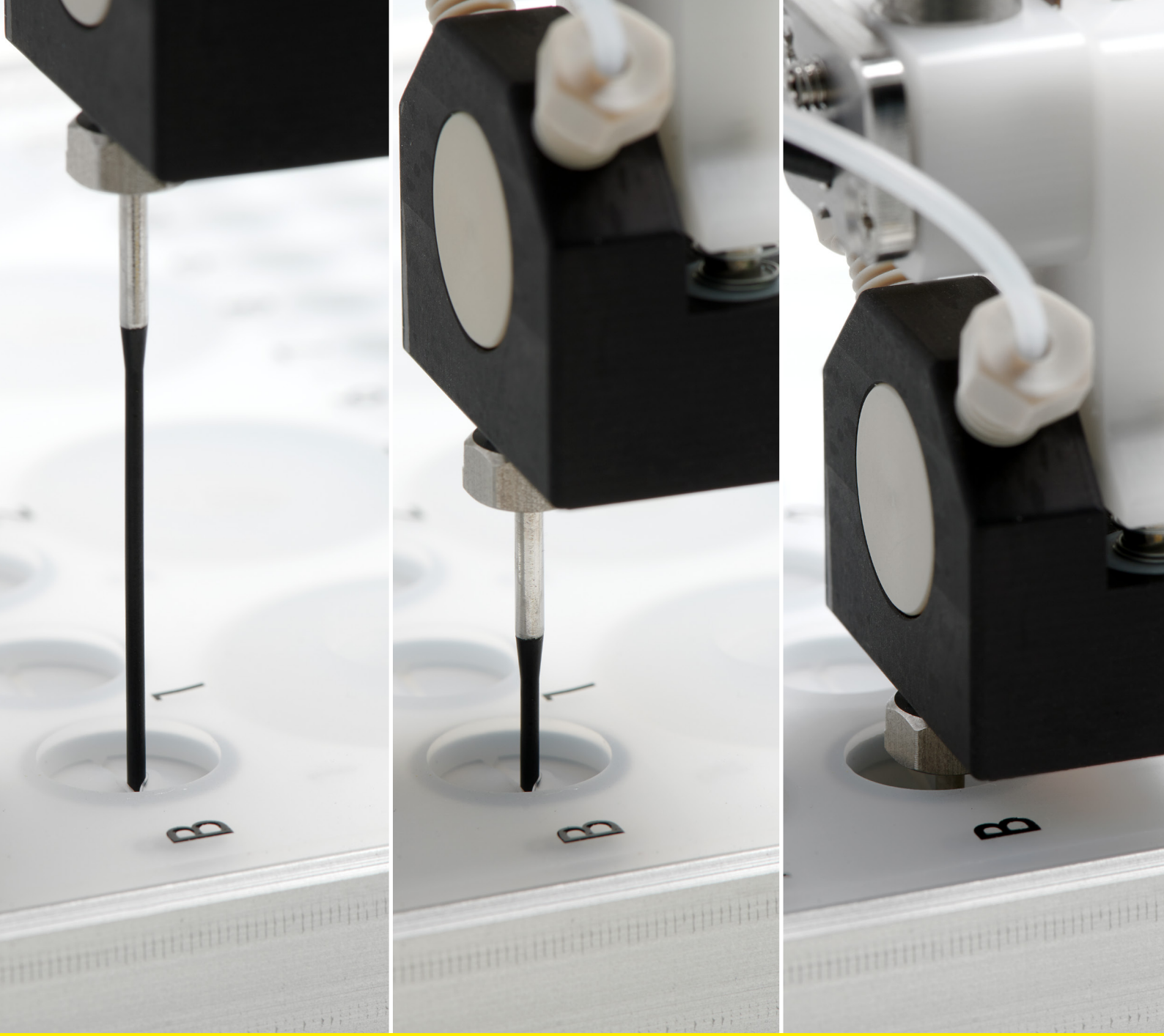
Simplifying Progress

SARTORIUS

Powered and Prepared for Whatever Challenge You Take On

To keep pace with the current global demand for antibody fragment characterization, vaccine research and whole cell biologics, continuous technological advances are crucial in helping turn initial research hypotheses into actionable results. This is especially true within the field of label-free protein analysis.

With exceptional sensitivity for both small and large molecules, low baseline noise and drift, large injection volumes and the novel OneStep® and NeXtStep™ Gradient Injection Technologies, the new Octet® SF3 allows users to generate high-quality kinetics and affinity data in a fraction of the time compared to standard multi-cycle kinetics. Combined with software designed by users for users, the Octet® SF3 offers a robust, high-throughput, low maintenance SPR solution for the rapid characterization of a wide variety of biomolecular interactions.



Robust and Low-Maintenance SPR
State-of-the-art fluidics and optimized electronic designs significantly reduce the potential for instrument failure and blockages, minimizing instrument downtime and resulting in the generation of reliable, high-quality data.

High-Throughput SPR
Generate complete binding kinetics and affinity for up to 768 samples in a single unattended assay. The combination of OneStep® Injection Technology, more than 72 hours unattended run time and a unique sample layout allows for high-throughput acquisition and analysis of hundreds of samples in a single run.

OneStep® Injection Technology
Streamline assay development and performance by eliminating the need to prepare multiple dilution series by using OneStep® Injection Technology. Simply prepare a single analyte solution to create a comprehensive concentration gradient for kinetics and affinity analysis.

Competition Assays
Determine an analyte's full kinetics and affinity in the presence of multiple competitors from a single analyte concentration using NeXtStep™ Injection Technology.

The Octet® SF3: A Unique Blend of Innovation and Design

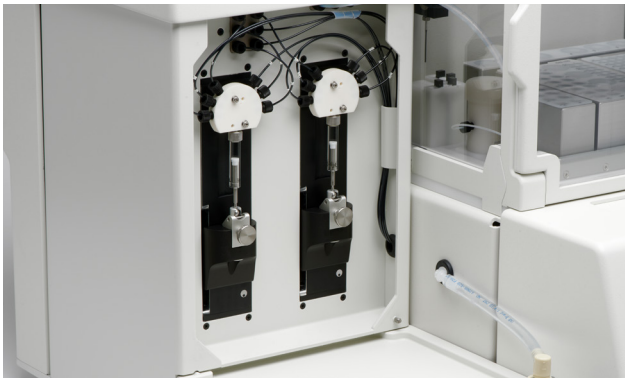
A unique combination of technological innovation, complemented by a robust engineering design, helps to make the Octet® SF3 the system of choice for both frequent and infrequent SPR users, offering low system maintenance, high sample throughput and a highly accurate characterization of both small and large molecules.

Key advantages include:

- Determine accurate kinetics and affinity using the unique OneStep® Gradient Injection which eliminates the need for multiple analyte concentrations, reduces surface regeneration injections and significantly reduces assay development time.
- High sensitivity sample measurement for both small and large molecule assays.
- High precision over a broad range of kinetic rate constants allows the measurement of even the highest affinity interactions with confidence.
- NeXtStep™ Gradient Injections for highly accurate competition assays.

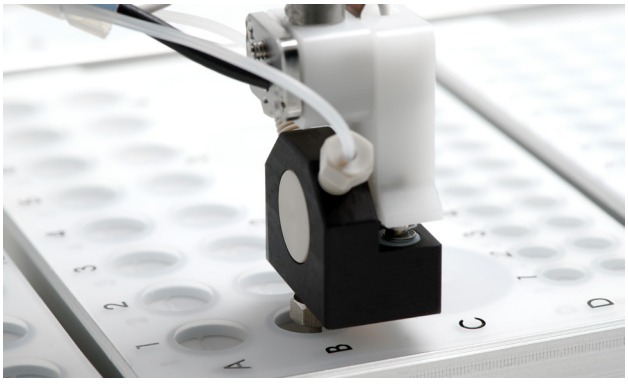
1 **Increased Buffer Volume**
The buffer tray can hold up to three one-liter bottles, comprised of one water and two buffer lines. Both buffer lines function independently, allowing two running buffers to be used during an assay.

2 **Optimized Fluidics**
All aspects of the Octet® SF3 fluidic system have been designed and engineered to minimize any potential sources of blockage, resulting in a strong, robust, low maintenance system. System desorb, clean, and decontaminate protocols ensure maximum system up-time.

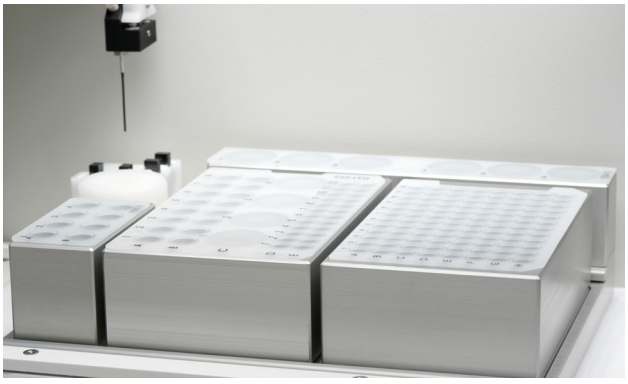


3 **Dedicated Water Line**
The Octet® SF3 contains a dedicated water line to minimize the risk of buffer precipitation and blockage formation.

4 **Thermodynamic and Physiological Measurements**
The Octet® SF3 allows you to study interactions across a wide range of temperatures to rapidly assess binding kinetics and affinity of therapeutics at physiological temperatures. An in-line buffer degasser prevents air bubble formation and when combined with a large syringe volume (500 µl), allows the accurate calculation of dissociation rate constants.



5 **Sample Recovery**
Recovering precious samples for further analysis can rapidly decrease workflow times. The Octet® SF3 allows you to recover bound analyte using a predefined Sample Recovery Injection, which means you can assess the same analyte using alternative analytical techniques.



6 **High-Throughput Sample Acquisition**
Combining OneStep® and high-throughput modes allows 768 unique samples to be analyzed in less than 24 hours. With more than 72 hours of uninterrupted run time, this makes the Octet® SF3 suitable for even the most high-throughput library screens.

OneStep® Gradient Injections

Based on the Taylor dispersion theory, OneStep® Gradient Injections diffuse a single concentration of analyte into a moving stream of buffer to create an analyte concentration gradient of at least 3 orders of magnitude, allowing an accurate measurement of molecule kinetics and affinity from a single analyte concentration.

While standard Multi-Cycle Kinetics (MCK) can also be performed on the Octet® SF3, OneStep® Injections eliminate the need to prepare a dilution series of each analyte.

OneStep® Gradient Injection advantages include:

- **Accuracy:** negligible rate constant differences with multi-cycle kinetics.
- **Speed:** significantly less time required for sensor chip regeneration, sample preparation, acquisition, and analysis to obtain kinetics and affinity.
- **Throughput:** unlock the full potential of each sample plate as only a single analyte concentration is required to assess each interaction.

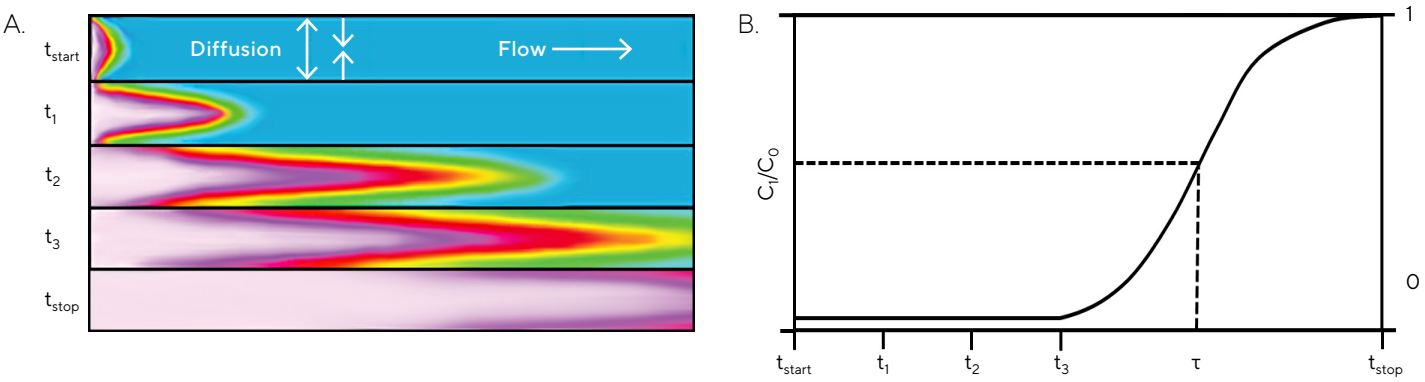


Figure 1

Note. (A) OneStep® gradient formation in the injection line, with (B) the corresponding analyte concentration measured within the flow cell. Blue color indicates the running buffer and pink color indicates the analyte. The gradient formation and its relationship to analyte concentration at the flow cell is illustrated using five simulated snapshots (t start – t stop) of the injection line at different times, and shows that a single injection can be used to assess a full analyte concentration series.

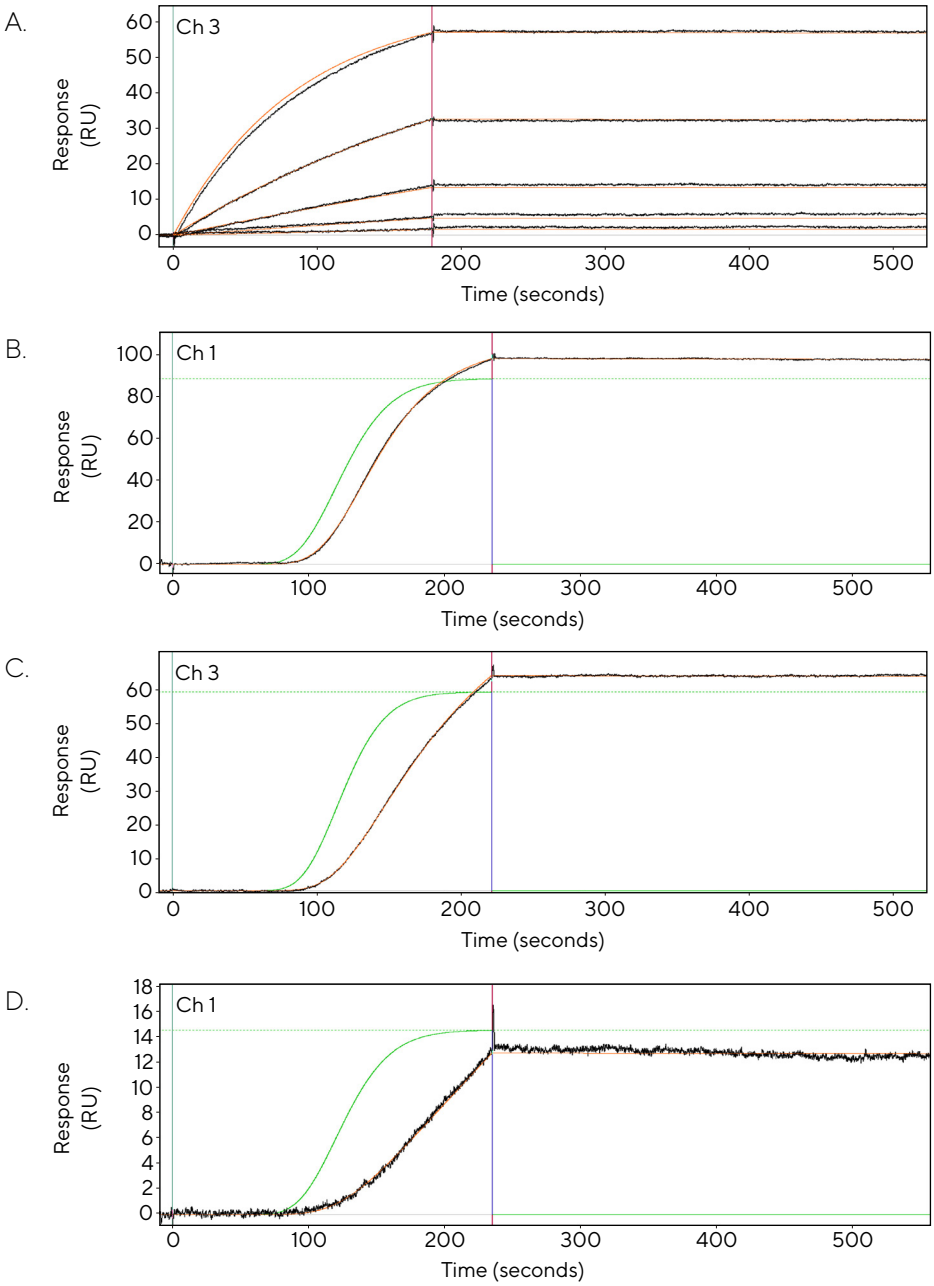


Figure 2

Note. Trastuzamab Fab antibody (analyte) against HER2 (ligand). Results were generated using both (A) multi-cycle kinetics (short-long, full dissociation not shown), and (B-D) OneStep® Injections on the Octet® SF3. Serial multi-cycle kinetic dilutions for the Trastuzamab Fab antibody were 25 nM, 8.3 nM, 2.78 nM, 0.926 nM and 0.309 nM.

Following the preparation, acquisition and global analysis of the 5 different Trastuzamab dilutions using standard multi-cycle kinetics, the global affinity constant (K_D) was calculated at 7.38 pM.

Using OneStep® Gradient Injections, no significant differences in K_D values were found at any of the concentrations prepared for the multi-cycle kinetic assays. For example, K_D values of 9.85 pM (figure 2B), 8.49 pM (figure 2C) and 8.60 pM (figure 2D) could be achieved using Trastuzamab concentrations of 25 nM, 8.3 nM and 0.926 nM, respectively.

Conclusion

The use of a single analyte concentration using OneStep® Gradient Injections resulted in similar results compared to those generated using a serial dilution multi-cycle kinetic assay. Significant time, cost and labor savings, however, can be achieved for high-throughput hit identification screens, as well as in everyday instrument use.

NeXtStep™ Gradient Injections

Competition assays are very useful in drug discovery, yielding the ability to find active site binders by competing fragment hits with a control molecule. NeXtStep™ Injection Technology has the unique ability to determine the behavior of an analyte in the presence of a competitor molecule from a single injection. Since the competitor does not need to be present in the running buffer, NeXtStep™ allows you to assess multiple analytes and competitors in a single assay. Full kinetic profiles, affinity and site-specific competition can be clearly identified as a modulation of binding in the presence of the competitor molecule.

NeXtStep™ Gradient Injections comprise of solutions A (analyte and competitor molecule) and B (competitor molecule only), with the competitor molecule concentration kept constant throughout. Both solutions are sequentially diffused into the sample holding line, where upon reaching the flow cell, the order of the solutions is inversed. The first part of the injection consists almost entirely of solution B (Fig. 3). As the NeXtStep™ Injection progresses and the concentration of the competitor molecule remains constant, the analyte concentration increases in a gradient fashion (Fig. 4).

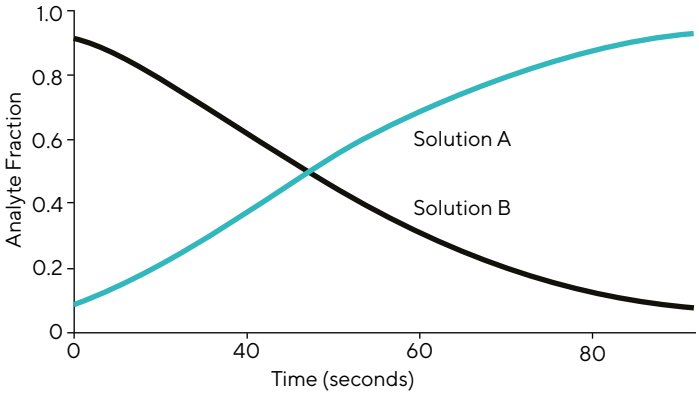


Figure 3

Note. Time course charting the fraction of each solution as it passes through the flow cell. At the beginning of the injection, the solution almost entirely consists of solution B (the competitor molecule), however as the injection progresses, it is gradually replaced by solution A (the analyte + competitor molecule), until the final stages of the injection, which consists primarily of solution A.

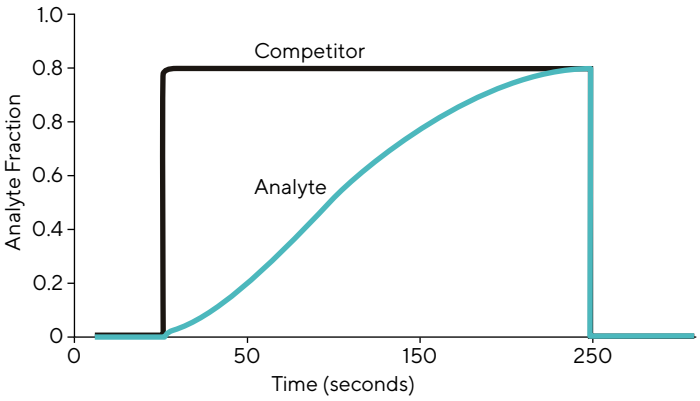


Figure 4

Note. The concentration of the competitor molecule remains constant throughout the assay. However, the concentration of the analyte slowly increases as solution A becomes more prominent during the course of the injection.

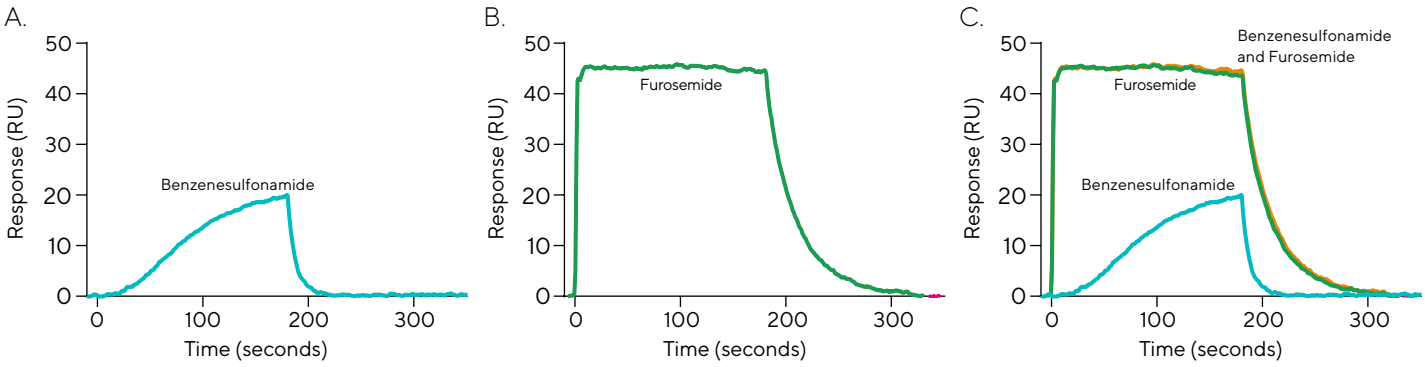


Figure 5

Note. NeXtStep™ Gradient Injections were used to identify any competitive effects in the binding of Furosemide and Benzenesulfonamide to biotinylated Carbonic Anhydrase II.

An Octet® SPR SADH Sensor Chip was prepared with biotinylated Carbonic Anhydrase II as the ligand. Successive NeXtStep™ Gradient Injections of (A) Benzenesulfonamide (blue), and (B) Furosemide (green) across the same sensor chip surface with HBS-EP+ in place of the respective competitor molecule were performed. (C) A competitive NeXtStep™ Gradient Injection using single concentrations of Benzenesulfonamide and Furosemide (as the competitor molecule) was then performed (brown line) and compared to the individual uninhibited binding profiles of Furosemide (green) and Benzenesulfonamide (blue).

Comparison of the Furosemide (green), Benzenesulfonamide (blue), Furosemide and Benzenesulfonamide (brown) sensorgrams indicate the absence of any additive response, with the higher affinity interaction of Furosemide fully inhibiting the binding profile of the weaker affinity Benzenesulfonamide. It is therefore likely that the two molecules share a common binding pocket, since an additive response would have been expected had each molecule been able to bind to the Carbonic Anhydrase II ligand individually.

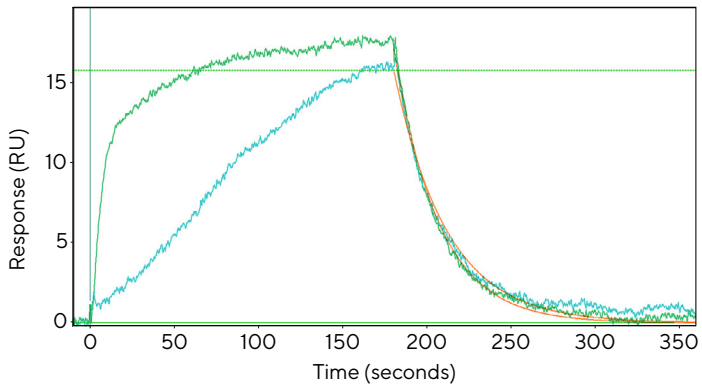


Figure 6

Note. NeXtStep™ Gradient Injection assays can be run as a direct competition assay between an analyte and a competitive molecule to assess any impact on binding.

As shown in Figure 6, Furosemide was injected with HBS-EP+ (green) to determine the kinetic response in the absence of a competitive molecule. A NeXtStep™ Gradient Injection was then performed with Methanesulfonamide as the competitor molecule, with a clear shift in Furosemide binding (blue).

A key feature of NeXtStep™ Gradient Technology is that multiple competitive molecules may be injected directly from the assay plate to identify any potential interaction with your target analyte, allowing a large number of competition assays to be rapidly performed within a single, unattended run.

Software Specifically Designed for Low Instrument Maintenance and Ease of Use

The high-quality data recorded on the Octet® SF3 system is easily analyzed using Octet® SPR Analysis software.

Assay Set-Up
With its intuitive drag and drop layout, assay design has never been easier. The Octet® SPR Discovery software allows you to rapidly define sample parameters, create common or independent sample flow rates, add injection volumes and report points. Generating data has never been easier.

The method setup page allows the user to select from numerous pre-written methods that help guide setup and provides the flexibility to write, modify, and view bespoke methods.

Rapid Data Analysis
Data analysis of SPR-based fragment screens can be cumbersome and require days of an analyst's time for a single plate screen. Octet® SPR Analysis software uses a unified approach for the selection of hits from non-hits and allows normalizing of screening data across different days and instruments, so an entire screen campaign can be compared rapidly. This integration results in a major reduction in post-processing time — from hour or days, to seconds or minutes.

The software provides models incorporating kinetics, affinity, mass transport corrections, and multi-site binding as required to fit the interaction. Primary screening data are ready for K_D analysis without having to perform a laborious, time consuming, and potentially error-prone secondary screening.

Maintenance
Maintenance of the Octet® SF3 requires minimal user interaction, with less time spent on maintaining your system and more time focussed on generating data.

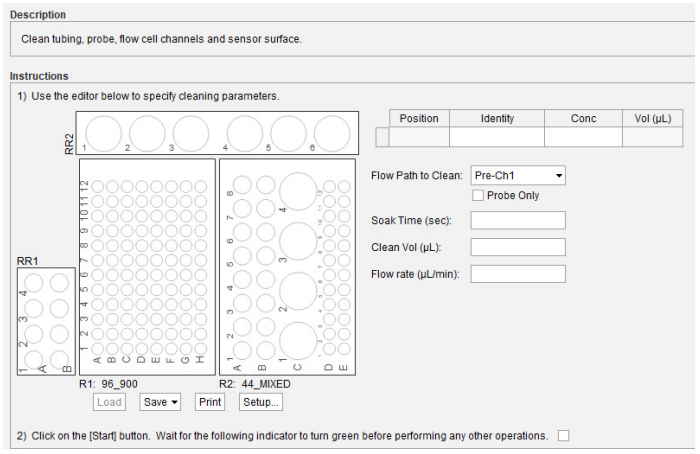


Figure 7

Note. Octet® SPR Discovery Software: Instrument tubing, probing, flow cell and sensor surface clean menu to maximize instrument run-time despite requiring low user maintenance.

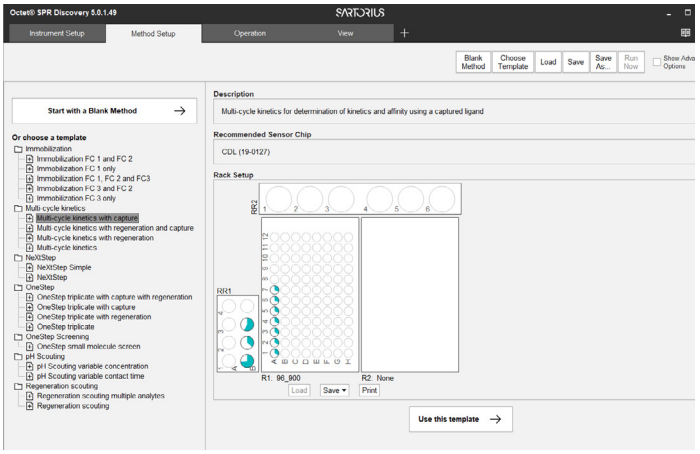


Figure 8

Note. Octet® SPR Discovery Software: Pre-defined method protocols for all potential assays to aid in the easy generation of high quality data.

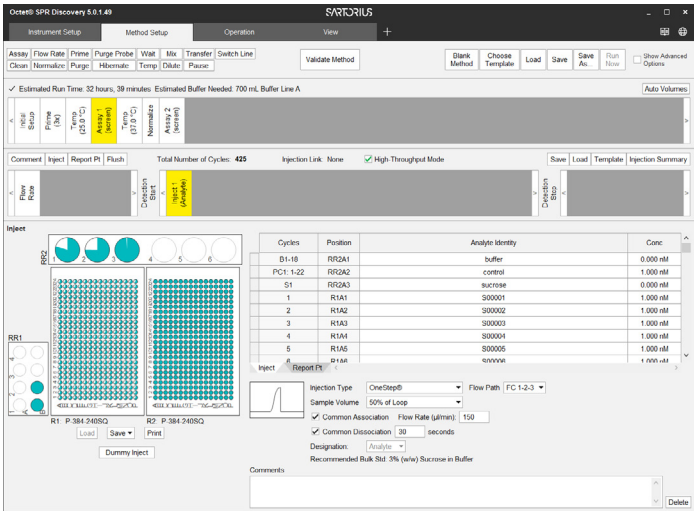


Figure 9

Note. Octet® SPR Discovery Software: Intuitive layout of the assay method set-up page includes variables such as analyte identity, concentration, injection type and common association and dissociation times.

Octet® SF3 Ordering

Octet® SF3	Product Number
Octet® SF3 SPR System	Octet-SF3
Octet® SF3 SPR System Installation	84IAFF3

Octet® SF3 Specifications

Baseline Properties	
Refractive Index Range	1.33 – 1.40
Baseline Noise	Typically < 0.025 RU (RMS)
Baseline Drift	Typically < 0.3 RU/min
Molecular Weight Cut-Off	No lower limit for organic molecules
Variable Data Rate	1, 2, 5, 10, 20 and 40 Hz
Affinity Range	fM to mM
Working Ranges	
Association rate constant (k_a)	$10^2 – 10^9$ M^{-1} s^{-1}
Dissociation rate constant (k_d)	$10^{-6} – 2.5$ s^{-1}
Global affinity constant (K_D)	$10^{-3} – 10^{-12}$ M
Concentration	Sample concentration >1 pM
Sample Acquisition	
Unattended Run Time	>72 hours with no built in run-time limit
Sample Capacity	Any 2 sample racks plus 2 reagent racks
Sample Rack Options	96 vial, deep well and PCR formats, 384-well microplates, custom high volume
System and Sample Temperature Control	4 to 40°C (max 15° below ambient)
Buffer Line Selection	Automatic switching between 3 independent lines
Inline Buffer Degasser	Yes
Flow Cell and Injections	
Number of Flow Channels	3
Flow Path	1, 1–2, 1–2–3, 3, 3–2, 3–2–1
Flow Channel Volume	<90 nL
Channel-Channel Dead Volume	<20 nL
Injection Volume	2-700 µL
Flow Rate	0.1–200 µL/min
Injection Rise and Fall Time	< 0.75 second @ 25 µL/min
Gradient Injections	OneStep® (including in High-Throughput Mode), NeXtStep™
Simultaneous Injections	Yes
Software	
Octet® SPR Analysis Hit Selection	Yes
Dimensions	
Width x Height x Depth	24" x 24" x 20" (61 cm x 61 cm x 51 cm)
Weight	66 kg

North America
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
USA
Phone +1 888 OCTET 75
or +1 650 322 1360
Email: orders.US07@sartorius.com

Europe
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Germany
Phone +49 551 308 0
Email: euorders.UK03@sartorius.com

Asia Pacific
Sartorius Japan K.K.
4th Floor, Daiwa Shinagawa North Bldg.
1-8-11, Kita-Shinagawa 1-chome
Shinagawa-Ku
Tokyo 140-0001
Japan
Phone +81 3 6478 5202
Email: orders.US07@sartorius.com

Find out more: www.sartorius.com/octet-support

For questions, email: octet@sartorius.com

Octet-SF3-Instrument-Brochure-en-L-Sartorius Status: 06 | 2022