SARTORIUS

Application Note

Scope
This application note introduces Sample Recovery Injections on the Octet® SF3 SPR system along with guidance on its application and implementation in an assay setup.

Keywords or phrases:
Octet® SF3, Surface Plasmon Resonance, Analyte Recovery, Ligand Fishing, Sample Recovery

Recovery of Sample Using the Octet® SF3

Stuart Knowling, Ph.D, Sartorius, Fremont, CA

Correspondence
Email: octet@sartorius.com

Abstract

Sample Recovery Injections allow users to identify molecules that possess desirable characteristics and then recover the sample so that successful candidates may be tested for critical quality attributes (CQA) and in further downstream analysis. Sample recovery is especially powerful in workflows such as Cell Line Development where early identification of high productivity clones with optimal CQA and target specificities can significantly shorten timelines and lead to substantial cost savings.

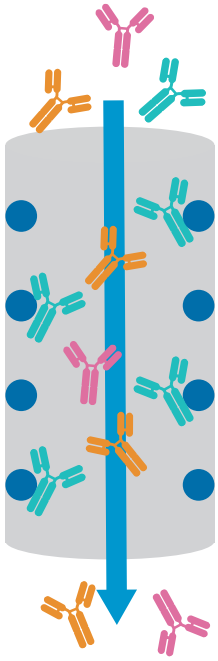
The Octet® SF3 offers a special type of injection for eluting and recovering a bound analyte from a sensor surface called, Sample Recovery Injection. In a sample recovery workflow, molecules of interest are passed over target ligand(s) during a first injection and then, in a second Sample Recovery Injection, returned to a user-specified location in the sample racks, ensuring that precious sample is not wasted and can be used for orthogonal studies.

Introduction

Surface Plasmon Resonance (SPR) assay setup shares many common features with affinity chromatography in that both utilize an immobilized molecule (ligand) to capture a target molecule (analyte) from a simple or complex solution. The target molecule containing the desired binding properties is isolated from all other molecules in the solution, which simply wash through to waste. The main difference between an affinity chromatography and SPR assay setup is that in affinity chromatography, the captured molecule is eluted from the column and collected for further analysis while in standard kinetics and affinity SPR assays, after measurement of the association and dissociation phase, the analyte bound to the ligand is removed to waste during a regeneration step and therefore, not usable for further orthogonal analysis (Figure 1).

The Octet® SF3 allows the addition of a simple assay step called Sample Recovery Injection, which allows users to efficiently recover a captured analyte to a separate vial or microplate. SPR is a non-destructive technique and the ability to recover samples of interest from simple or complex solutions (Ligand Fishing) for further downstream analysis can be highly beneficial. This can be especially useful where only small initial quantities of the molecule of interest are available and recovery may allow additional CQA to be identified, for example by mass spectrometry (MS). As with affinity chromatography, SPR can be used to detect a wide variety of molecular formats and sample recovery is not limited to protein samples; any analyte that binds a cognate ligand can be recovered using a Sample Recovery Injection, which includes but is not limited to; DNA/RNA and small molecules.

A



B

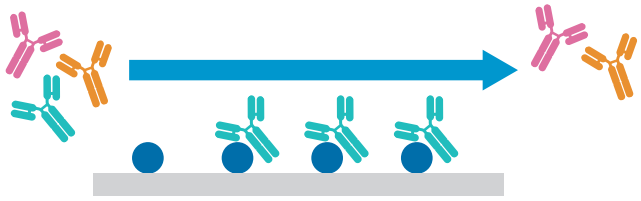
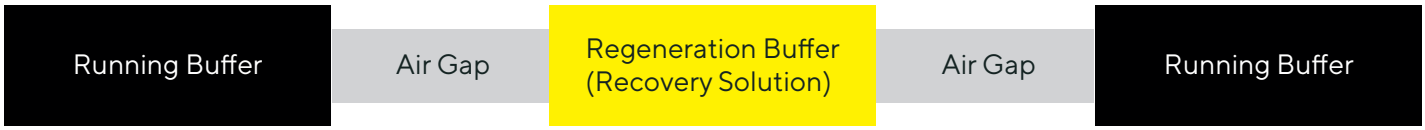


Figure 1: Affinity Chromatography and SPR Contain Common Elements. (A) Affinity chromatography is a purification technique that utilizes the reversible specific binding interaction between an immobilized ligand (blue circle) and its binding partner (teal antibody) presented in the mobile phase (B) Surface plasmon resonance also utilizes the ability of an immobilized ligand to capture its binding partner from the mobile phase.

Sample Recovery Injection – Assay Design Considerations

The Octet® SF3 allows users to capture analyte molecules from simple and complex solutions using standard injections and the captured analyte can then be recovered by simply inserting a second Sample Recovery Injection as shown in Figure 2. The Sample Recovery Injection requires the use of a regeneration solution (recovery solution) that must be optimized in advance but is oftentimes the same regeneration solution that is used in standard kinetics and affinity assays.

A



B

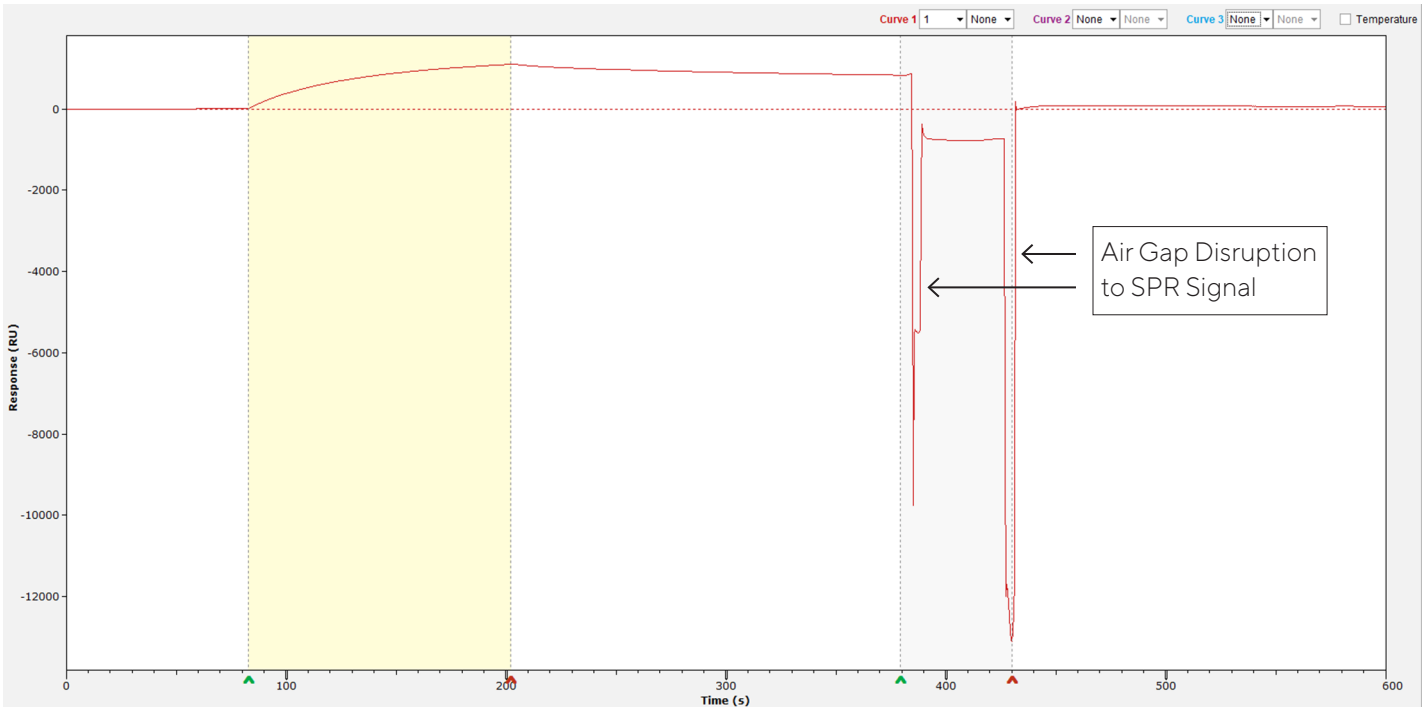


Figure 2: Sample Recovery Injection. (A) Pictorial representation of Sample Recovery Injection. A user-specified volume of the recovery solution that is contained within two air gaps is injected across the flow cell(s) to recover the bound analyte. (B) Example of a Sample Recovery Injection. Following standard analyte capture (yellow box), a recovery solution bookended by air gaps is injected over the desired flow cell(s) (grey box). The air gaps cause a momentary decrease in the SPR signal as shown and recovered analyte is deposited in a user-specified position in the sample rack.

As the recovery solution is injected, an air gap passes through the flow cell to prevent dispersion of the recovery solution into the system buffer. Once the recovery solution reaches the flow cell, the flow can be paused for a configurable amount of time, which allows removal of the captured analyte from the sensor chip surface (**See Effect of Soak Time**).

After any soak is completed, the recovered analyte is withdrawn from the flow cell along with the air gap (thus the signal decrease at the beginning and end of the recovery injection, Figure 2B). The recovered analyte is then dispensed to a user-specified recovery position in the sample rack in either a vial or microplate.

As a recovery solution is required to elute the analyte, it may be beneficial for downstream analysis for the recovery position to contain a neutralization or deposition buffer in order to minimize possible damage from harsh regeneration conditions or initiate desired assay next steps such as enzymatic digest. Neutralization buffer may be added manually or can be automated using the **Transfer** and **Mix** functions available on the Octet® SF3 (Figure 3).

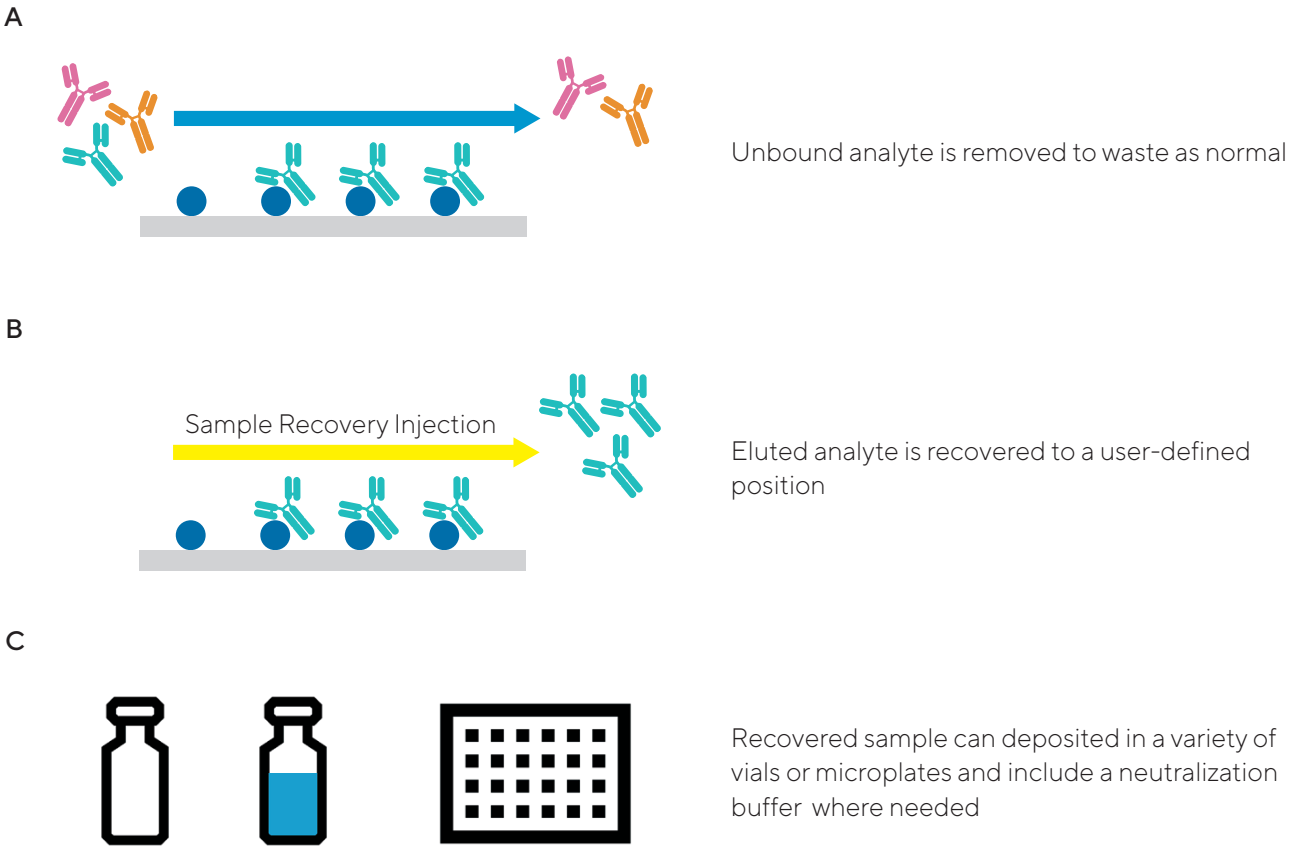


Figure 3: Example Workflow of a Sample Recovery Injection. (A) Simple or complex mixture of analyte can be injected over an immobilized ligand and only those that contain the desired binding properties are retained, whilst unbound material is washed through to waste as standard. (B) A Sample Recovery Injection is performed to elute the bound material from the ligand and the Octet® SF3 system removes the eluted material to a user-specified vial or microplate in the sample rack. The ligand may then be reused for additional analyte capture to increase the amount of a single analyte or capture other molecules with desired properties. (C) The Octet® SF3 allows the use of a variety of vial and microplate formats to be used to accept the recovered analyte. Where harsh recovery solutions are required to remove the bound analyte, a neutralization buffer may be added to the receptacle either manually before the assay or by use of the **Transfer** command in the Octet® SPR Discovery Software. The Octet® SF3 also allows efficient neutralization of the recovered analyte in the neutralization buffer to be automated through use the **Mix** command contained within the Octet® SPR Discovery Software.

An important consideration in the success of a Sample Recovery Injection is that the amount of analyte recovered will normally be a critical factor for successful downstream analysis. In contrast to standard kinetics and affinity assays, where low ligand immobilization levels are prioritized in order to minimize mass transport limitation (MTL), Sample Recovery assays should prioritize high levels of ligand immobilization, which in turns ensures maximum analyte binding.

To ensure optimal analyte binding, the sample should be concentrated where possible and low flow rates prioritized (<10 µL/min).

Where high levels of sample recovery are necessary multiple flow cells can be immobilized with the ligand and analyte captured on all flow cells prior to Sample Recovery (**See Sample Recovery Quantitation**). The Octet® SF3 contains a high surface capacity due to its longer fluidic paths than competitor SPR systems, which makes it ideally suited for Sample Recovery Injections. If the quantity of recovered analyte is still too low after increasing the number of flow cells, it is recommended that multiple replicates of Sample Recovery Injections are performed and deposited in the same vial or well on a microplate (**See Sample Recovery Injection Setup**).

When working at such high immobilization and capture levels, non-specific binding (NSB) may be experienced. This non-specific binding may cause undesired analyte to be carried over into the Sample Recovery Injection and therefore, NSB should be experimentally determined using a blank sensor chip prior to sample recovery assays. Where NSB is experienced on a blank chip, standard measures can be taken to reduce the level (**See Best Practice Guide: Minimizing the Effects of Non-specific Binding**). As the Octet® SF3 allows you to address specific flow cells for Sample Recovery Injections the flow cells preceding the flow cell contained the immobilized ligand may be used to help reduce any NSB carryover into the Sample Recovery Injection.

Methods

Instrument and Reagents
All assays were performed using an Octet® SF3 SPR system. Hepes buffered saline with 0.05% Tween 20 (HBS-EP+), pH 7.4 was used as running buffer throughout. Unless indicated, all assays were performed at 25 °C. Mouse IgG was purchased from Athens Research. All other reagents were purchased from Sigma Aldrich and prepared in-house.

Sensor Chip and Reagent Preparation
Biotinylated Protein A was prepared to 50 µg/mL and subsequently captured onto flow cell 1 of an Octet® SPR SADH Sensor Chip using a flow rate of 10 µL/min. Approximately 1200 RU of Biotinylated Protein A was captured on flow cell 1.

Next, mouse IgG was prepared to a final concentration of 50 µg/mL in HBS-EP+ and used for sample recovery injections. 20 mM NaOH was used as the recovery solution for all regeneration injections. The sample rack was sealed using resealable septa and placed in the sample tray set to 15 °C.

Sample Recovery Injection Setup

In the Method Setup, click on **Blank Method** and then drag in a Multi-cycle kinetics assay with regeneration into the Method Editor.

Sample Recovery Assay Recommendations
Assay Settings
- Cycle Order: Sequential
- Sampling Rate: 1 Hz
- Replicates: 1 (where larger amount of sample are required, increase replicates as required)
- Blank Cycles: None
- Startup cycles: None
- Pos Control Cycles: None
- Neg Control Cycles: None
- Bulk Std. Cycles: None

As shown in Figure 4 below, a Sample Recovery Injection requires three components, an analyte to be captured (mouse IgG), a recovery solution (20 mM NaOH) and a recovery position for the recovered analyte. Here, the 96 well reagent rack (96_900) and 500 µL microwell plate (P-96-450R) have been configured.

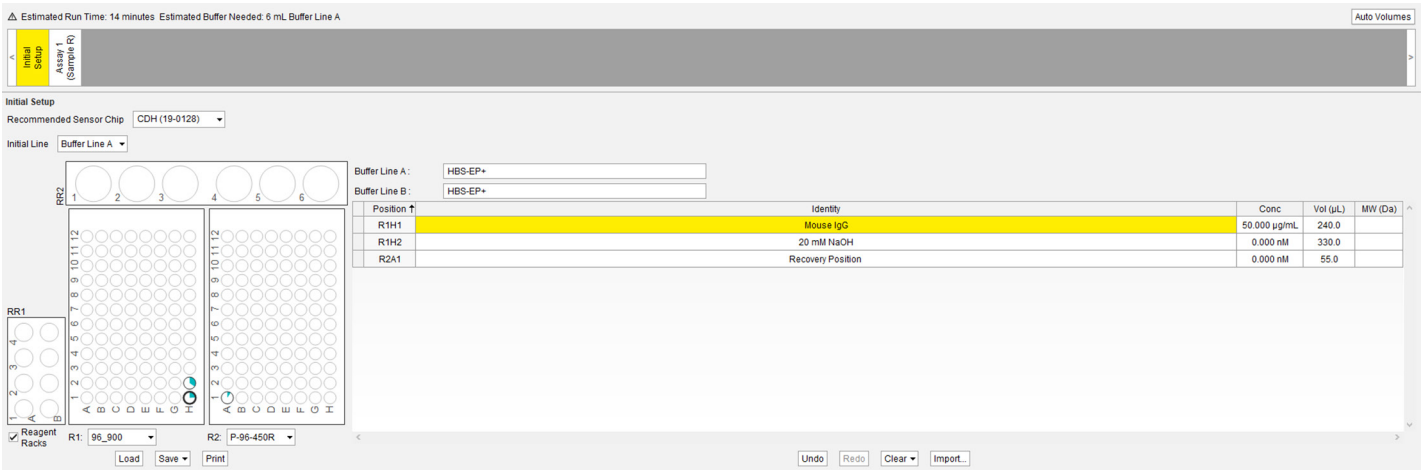


Figure 4: Initial Rack Setup. The Octet® SF3 allows multiple combinations of sample racks and microplates to be used for recovery injections, allowing up to 384 unique analytes to be assessed and recovered in a single unattended assay.

In the cycle editor the capture injection of the mouse IgG is configured as shown in Figure 5.

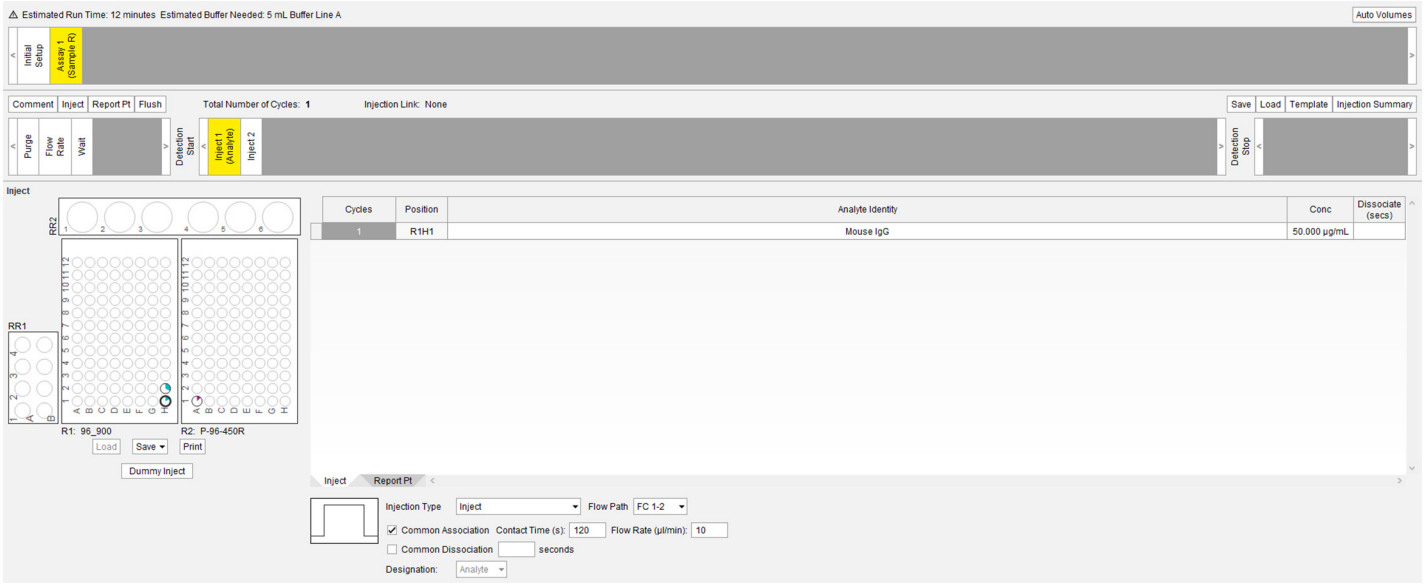


Figure 5: Analyte Capture Settings. Analytes for assessment are captured using standard injections across user-specified flow cells. The location of the analyte in the sample rack is designated by dragging it to the sample table. Where multiple analytes are to be captured, their positions can simply be dragged onto the sample table to form further capture cycles after each Sample Recovery Injection. In order to minimize analyte dissociation from the ligand before the Sample Recovery Injection, dissociation time should be left empty.

The Sample Recovery Injection is configured in Inject 2 (Figure 6). The Injection Type must be set to 'Recovery' for the sample recovery position box to appear. The recovery solution should be dragged to the first 'Position' box and the desired sample recovery to the second 'Recover Pos' box. Where a soak time is desired, this parameter can be entered on the right-hand side (**See Effect of Soak Time**). Here, a contact time of 15s at 50 µL/min will return an approximate recovered volume of approximately 12.5 µL. Where any additional steps are required prior to sample recovery, such as Purge, Clean or a wash step, these may be simply defined by dragging an Inject command prior to the Sample Recovery Injection.

When the method is ready for checking, click the **Validate Method** button to ensure there are no critical errors. Make note of the assay run time to ensure the ligand(s) and samples are active for that length of time and that the buffer is available in sufficient quantity and stability for the full run time.

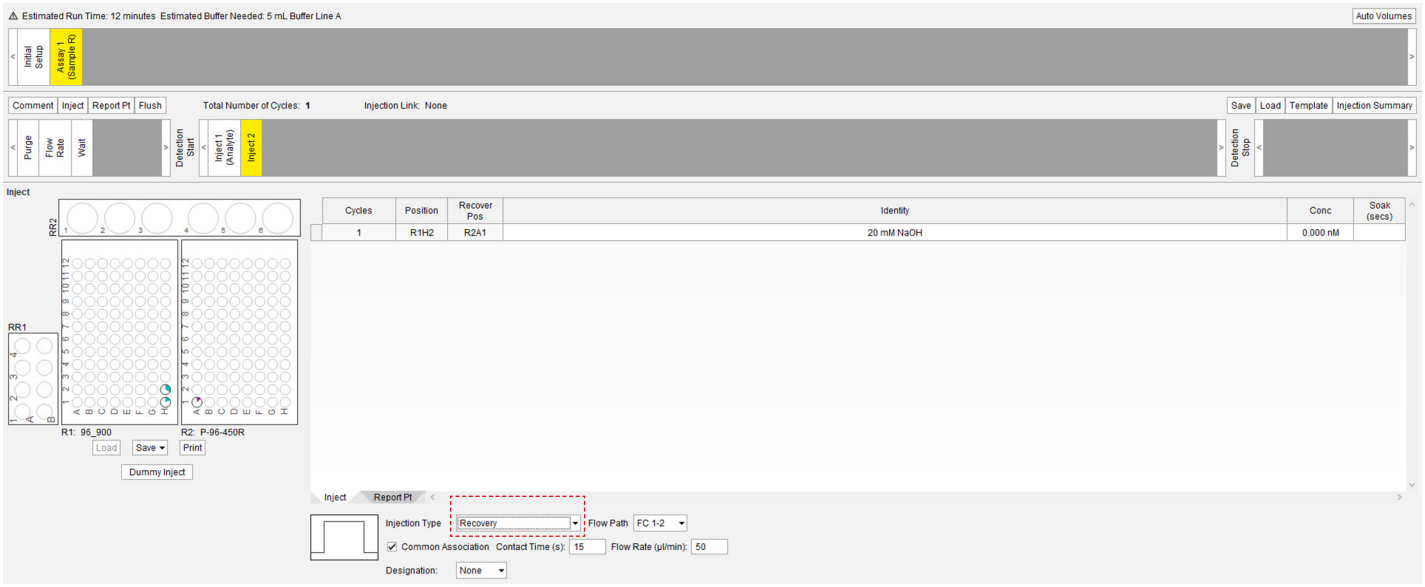


Figure 6: Sample Recovery Injection Settings. The location of the recovery solution in the sample rack is designated by dragging it to the Position box, the user-specified recovery position (to deposit the analyte) is designated by dragging the desired position to the Recover Pos box. Where desired, a soak time of the recovery solution can be entered in the Soak box.

Results

As shown in Figure 7, approximately 1000 RU of the mouse IgG was captured onto the Protein A surface prior to the recovery injection. As expected, the response returned to baseline after the Sample Recovery Injection and further capture and recovery can be performed. Where the response does not return to baseline a longer contact time may be required or a soak period can be used.

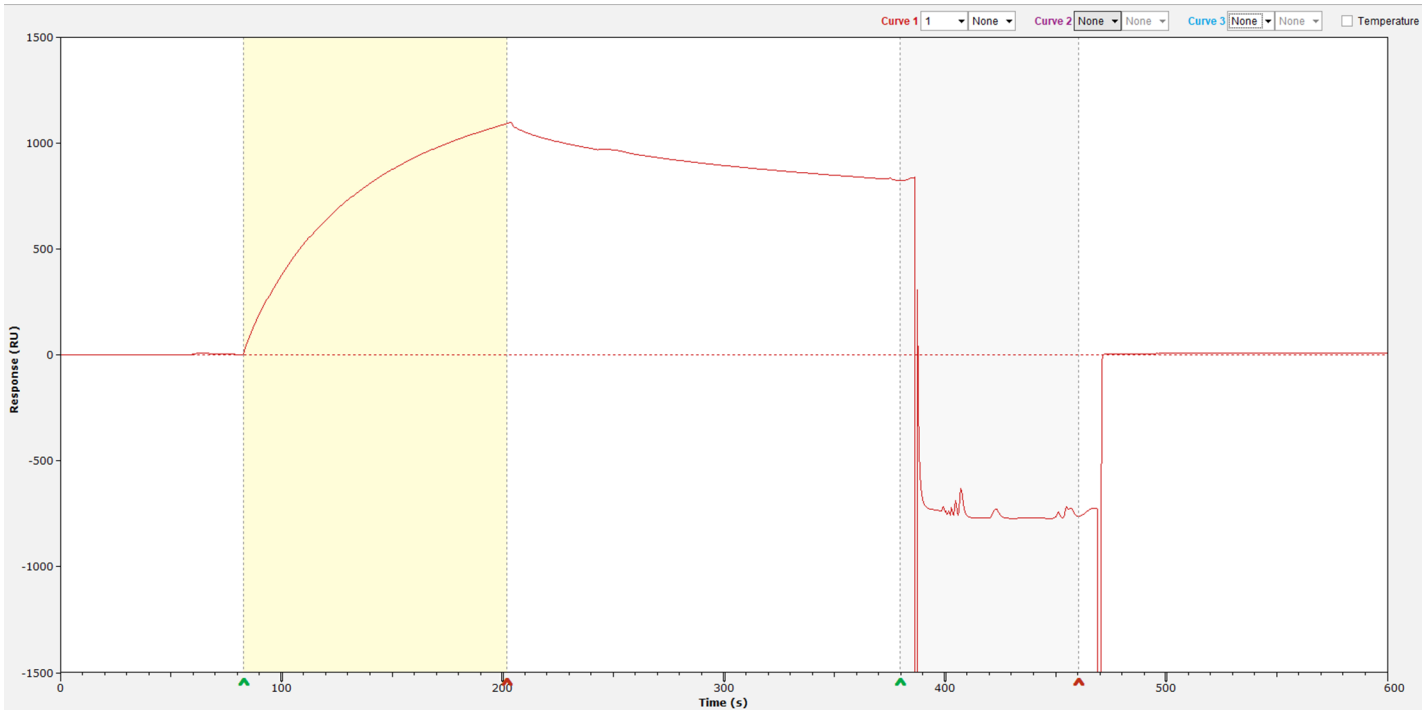


Figure 7: Sample Recovery Injection Removes Bound Analyte to a User-specified location. Use of a standard regeneration solution as the recovery solution (20 mM NaOH), flow rate and contact time for Protein A is suitable as a recovery solution for mouse IgG.

Effect of Soak Time

As discussed in previous sections, soak time allows the user to determine a final desired elution volume that the analyte is recovered in without having to alter any flow rate or contact time variables. For example, in the overlayed data in Figure 8 the effect of increasing soak time can be observed in that the Sample Recovery Injection with no soak time does not fully remove all analyte captured on the Protein A surface but increasing the soak time to 30 seconds or 60 seconds does cause a return to baseline and thus a higher concentration of analyte in the recovered volume without altering the flow rate and or contact time of the recovery solution.

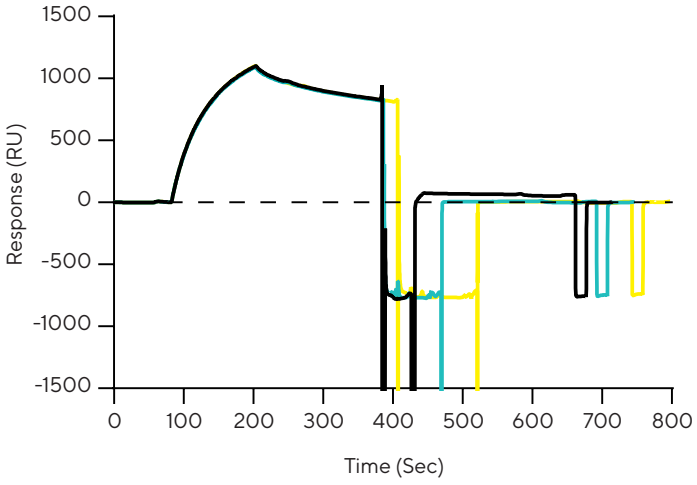


Figure 8: Increasing the Soak Time Allows more Sample Recovery in the same Volume of Recovery Solution. Capture of the mouse IgG show excellent reproducibility across injections but as shown, a Sample Recovery Injection with no soak time (black) is not sufficient to remove bound analyte from the surface. Increasing the soak time without changing the flow rate or contact time allows complete recovery of the analyte with a 30 second soak time (teal) or 60 second soak time (yellow).

Sample Recovery Quantitation

The quantity of analyte recovered during a Sample Recovery Injection can be estimated from the response unit displayed in the sensorgram. An approximate correlation of 1 RU corresponding to 1 pg/mm^2 can be used. In the example shown in Figure 7, after the capture phase a decrease to 850 RU is observed before the Sample Recovery Injection and therefore, approximately 850 pg/mm^2 is available for recovery, which equates to ~3 ng of protein. With an approximate recovery volume of 12.5 µL, a concentration of 240 ng/mL (1.6 nM for a 150 kDa antibody) is the maximum concentration expected from this Sample Recovery Injection.

Where sample recovery is sufficient, re-injection of the sample can be used to quantify the recovered amount or determine its binding potential to another target. Although differences in the refractive index of the running buffer and recovery solution should be considered along with the need for possible neutralization of the recovery buffer and subsequent decrease in concentration of the recovered sample.

Conclusions

As described above, the Octet® SF3 Sample Recovery Injection offers a simple method for the recovery of analyte bound to a target ligand. Thanks to the large surface area offered by the Octet® SF3 sensor chip flow cells, a large amount of analyte can rapidly be recovered for further orthogonal analysis. Unlike competitor SPR systems, Sample Recovery Injections on the Octet® SF3 can be performed across individual flow cells or across a combination of flow cells to increase sample recovery where necessary. This flexibility in assay design means that more data can be generated in a single unattended run as additional SPR assay can be performed to determine kinetics and affinity using OneStep® injections that require only a single analyte concentration (for example, a recovered antibody could be assessed against an antigen). In addition, the opportunity to perform competition assays directly from recovered samples or against a designated competitor thanks to NeXtStep™ or OneStep 2-component injections.

Combined with the flexibility to deposit the recovered analyte in industry standard microplates, the Octet® Sample Recovery Injection is an ideal tool to accelerate drug discovery workflows.

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 888 OCTET 75
Or +1 650 322 1360

For further information, visit
www.sartorius.com/octet-support