

Antibody screening with Biacore™ SPR systems



Background

Screening is a central step in the development of antibodies and other biomolecules. Large sets of candidates are evaluated to identify those with properties best suited for their intended application, which span research, diagnostics, and biotechnology. Antibodies and other biomolecules often exhibit high structural complexity and are typically produced in living systems. As a result, antibody screening is frequently performed using complex sample matrices like cell culture supernatants or phage display-derived preparations, and antibody concentrations are often unknown. Analytical techniques that enable direct characterization of biomolecular interactions under these conditions are therefore particularly valuable.

In the development of therapeutic or analytical antibodies, selecting candidates with appropriate kinetic and affinity profiles, specificity, and favorable biophysical properties is essential. These characteristics ultimately determine their performance in the intended application or as a drug. Antibody screening aims to identify cell clones that produce antibodies suitable for their intended purpose by evaluating their specificity and binding characteristics through interaction studies with a defined antigen. Obtaining kinetic information early in the screening process is a significant advantage, as it reduces redundant work on unsuitable clones.

Key information obtained from antibody screening typically includes:

- **Specificity:** which clones produce antibodies with the desired target specificity?
- **Binding characteristics:** which clones display kinetic and/or affinity properties suitable for the intended application?
- **Relative expression level:** which clones exhibit a sufficient expression level for further development?

Screening approaches can be applied to a wide range of biomolecules, including full-length antibodies, antibody fragments (for example, Fab and scFv), multispecific antibodies, and recombinant proteins.

This application guide focuses on screening of monoclonal IgG antibodies. However, the same general principles apply to other molecular formats. Assay design and experimental conditions may need to be adjusted depending on the specific molecule and application.

Assay format

Antibody screening can be performed using two different assay formats:

Antibody as ligand and antigen as analyte

In most antibody screening applications, this format is implemented as a capture assay. Antibodies are captured on a surface via a generic capturing molecule rather than being covalently coupled. This approach is preferred when screening large panels of antibodies. The alternative, covalent coupling, requires individual coupling of each clone and consumes many sensor surfaces.

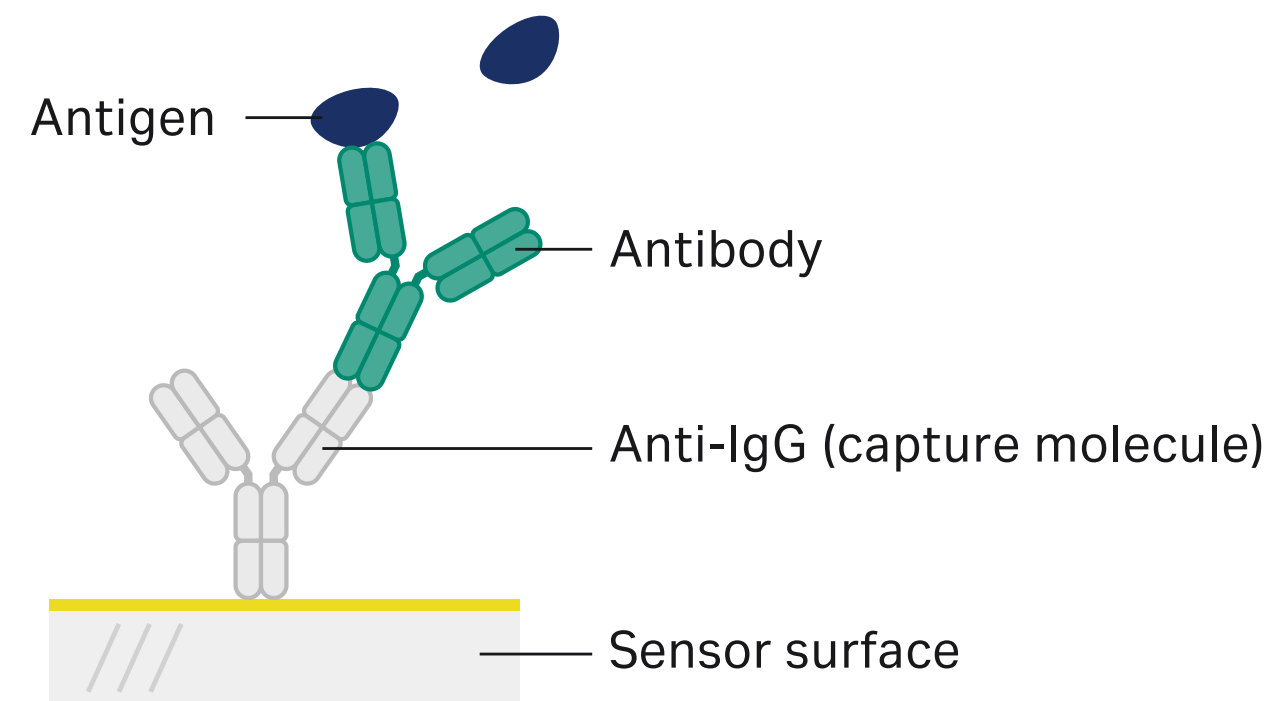


Fig 1. Antibody as ligand and antigen as analyte assay format.

In the antibody as ligand and antigen as analyte format, the generic capture molecule (for example a species-specific antibody) is coupled to the chip surface (Fig 1). Cell culture supernatants or phage display preparations containing antibody clones are injected over the surface and captured. Antigen is subsequently injected as analyte¹. The surface is regenerated by removing both captured antibody and bound antigen.

¹ In some Biacore systems, the analyte injection is termed sample. Do not confuse this terminology with the generic use of sample to refer to the variable element in the assay, in this case, the captured ligand.

Antigen as ligand and antibody as analyte

In this assay format, the antigen is either coupled to or captured on the chip surface. Samples to be screened are then injected as analytes (Fig 2). The surface is regenerated by removing bound antibody. If reversible antigen capture is used, the surface is regenerated by removing captured antigen and bound antibody.

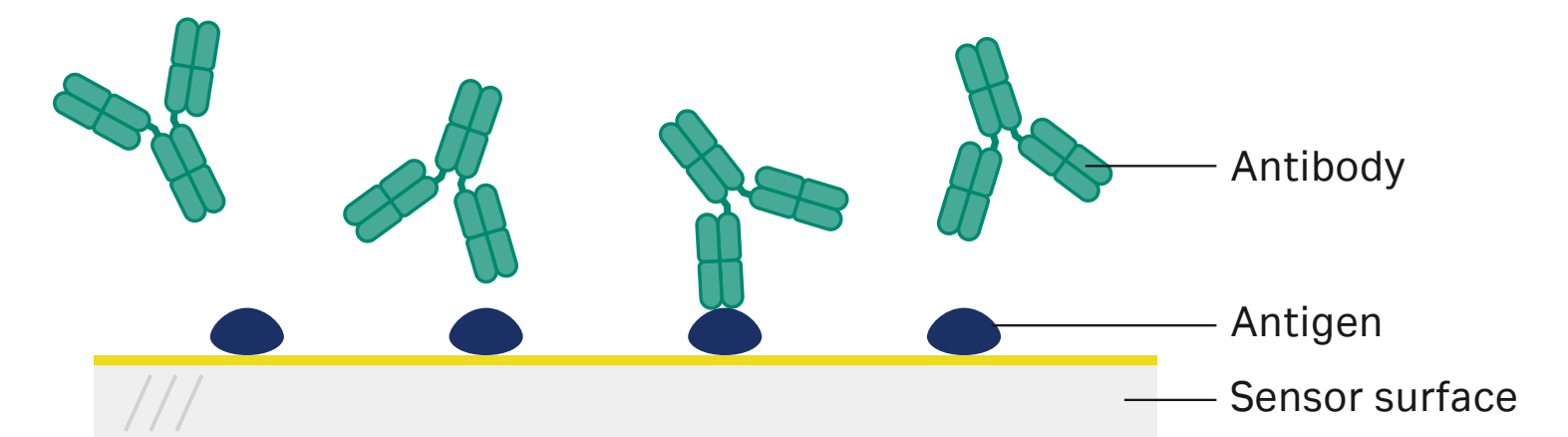


Fig 2. Antigen as ligand and antibody as analyte assay format.

Of these two approaches, capturing the antibody as ligand is generally more straightforward with respect to surface preparation and assay development. Capture kits with optimized protocols for coupling and regeneration are available from Cytiva. These assays are species or tag-specific but not antigen specific. They can therefore be re-used for many different projects that use the same species or tag for the screening molecule. However, the capture format requires injection of antigen in every cycle of the screen and relies on a stable antigen that does not change over the course of the assay.

The other approach, using the antigen as ligand, can involve additional work to establish coupling and regeneration conditions. This format is only recommended for assessment of specificity and/or expression level because:

- Antibody concentrations are usually not known, which is a requirement for kinetic characterization.
- Antibodies are usually bivalent, making evaluation of **Off-rate ranking** and **Kinetic screen** more difficult due to avidity effects.

The two approaches differ in experimental setup and evaluation and are described separately in the following sections.

Screening types

There are several screening types available for biomolecules using Biacore systems.

- **Kinetic screen**
- **Off-rate ranking**
- **Report point-based screening**

These screening types are described in more detail in the following section.

Kinetic screen

In **Kinetic screen**, cell supernatants or phage display preparations containing antibody clones are injected into the active flow cell over a surface functionalized with a species-specific or tag-specific molecule where antibodies are captured (Fig 3). A single concentration of antigen is then injected as analyte over both active and reference surfaces. The capture levels provide information on relative expression level. The antigen responses show the activity and specificity of captured antibodies and provide an additional indication of the active concentration of captured clones. From the shape of the association and dissociation of antigen, association and dissociation rates of the antibody-antigen interactions can be estimated to guide further selection of clones based on kinetic requirements. This type of screening requires that the antigen concentration is known and that each sensorgram is reference and blank subtracted.

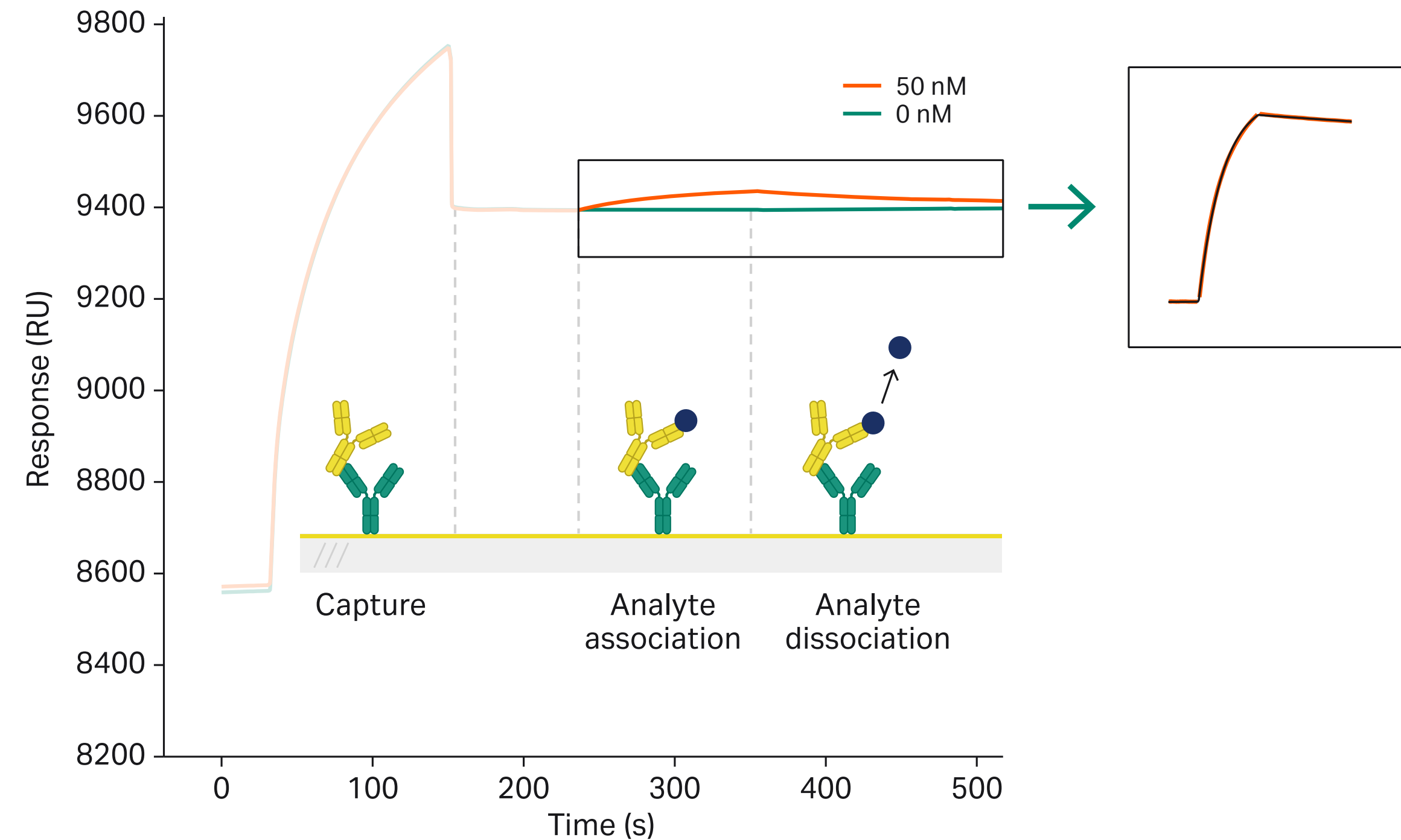


Fig 3. Schematic of **Kinetic screen**. Antibody clones are captured on the sensor surface and a single concentration of antigen is injected. A 1:1 binding algorithm is fit to the antigen association and dissociation phase.

Off-rate ranking

When antigen concentration is unknown, or when data quality in the association phase is inadequate for kinetic fitting, the same experimental setup as **Kinetic screen** can be used for **Off-rate ranking**. In this approach, a 1:1 dissociation model is applied to fit only the dissociation phase of the sensorgram (Fig 4). As for **Kinetic screen**, the capture level provides information on relative expression level. The antigen response reflects specificity and activity of captured clones. By fitting the dissociation phase to a 1:1 dissociation model, dissociation rate constants of the antibody-antigen complex can be assessed.

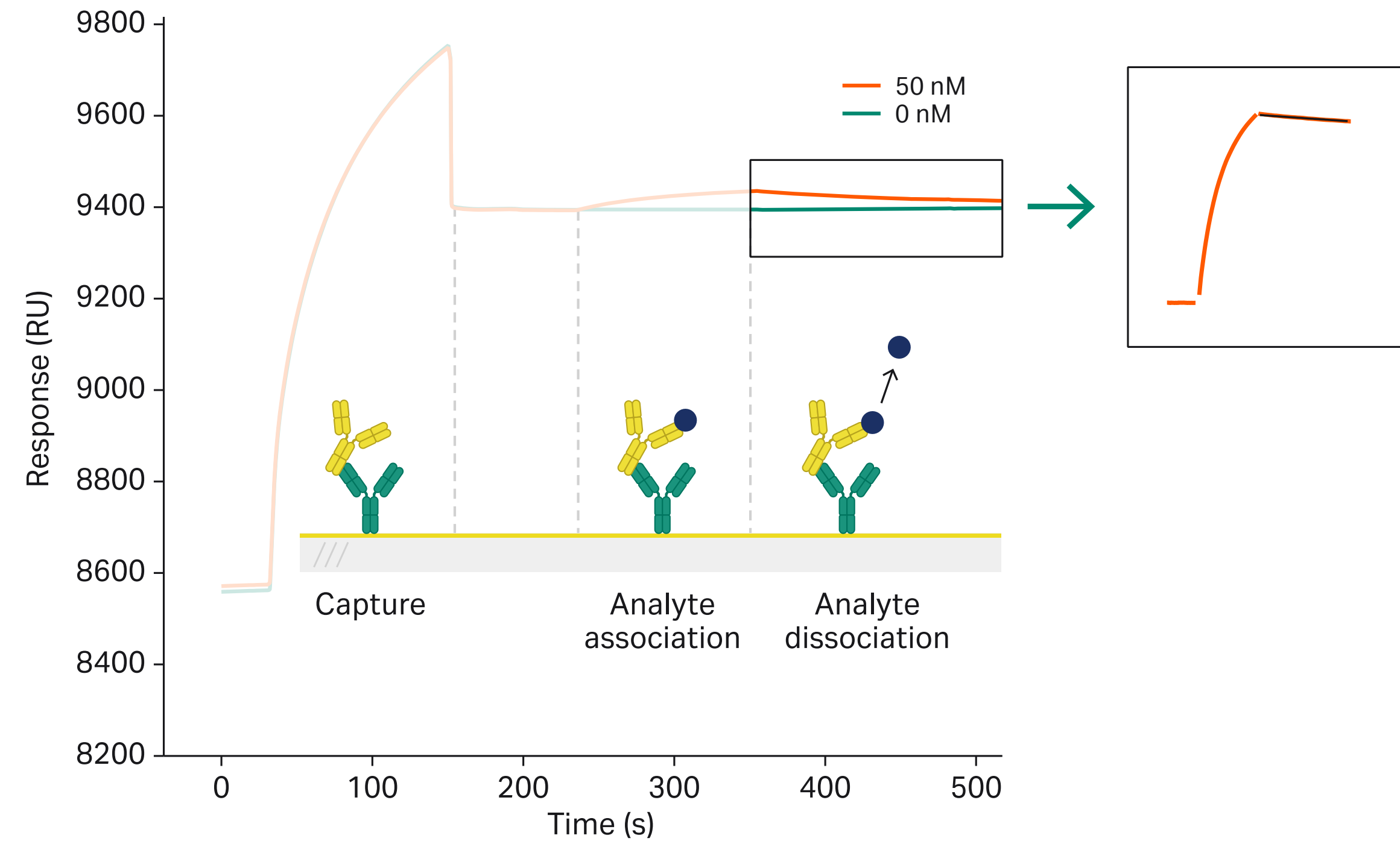


Fig 4. Schematic of **Off-rate ranking**. **Off-rate ranking** uses the same experimental setup as **Kinetic screen**. In **Off-rate ranking**, only the dissociation phase is fitted to a 1:1 model. This screening type is used when the antigen concentration is unknown or when data quality in the association phase is inadequate for kinetic fitting.

Off-rate ranking using 16 flow cells

As the name implies, this assay requires 16 flow cells arranged in eight parallel channels. In **Off-rate ranking using 16 flow cells**, a capture molecule is coupled in active and reference flow cells. For example, when testing 16 different clones, antibody clones 1 to 8 are injected into flow cell 1 (fc1), followed by injection of antibody clones 9 to 16 into flow cell 2 (fc2). A single concentration of antigen is then injected as analyte over both flow cells (Fig 5). This reduces the required amount of antigen by half. Since all flow cells are used

to capture the molecules to be screened, a blank cycle is required for each sample. The blank cycle consists of capturing the same antibodies followed by buffer injection instead of antigen. Blank subtraction removes capture-related drift, but injection-related disturbances such as bulk shifts may persist. Therefore, analysis of 16-flow-cell assays is limited to the dissociation phase, which is less affected by such disturbances. This assay provides the same information as **Off-rate ranking** performed using 8 flow cells.

Report point-based screening

For screening assays in which antigen serves as the ligand and antibodies act as analytes, kinetic parameters cannot be obtained because the exact concentrations of the screened molecules are usually unknown. In screens using full bivalent antibodies, this assay format carries a risk of introducing avidity effects, which complicates the evaluation of both **Off-rate ranking** and **Kinetic screen**. However, report-point analysis still delivers information regarding relative expression levels (from the capture levels), and the analyte responses provide information on specificity and activity of the molecules being screened.

For screening assays using antibody as ligand and antigen as analyte, report points (capture level, analyte stability early, and analyte stability late) can be used as a complement to kinetic analysis.

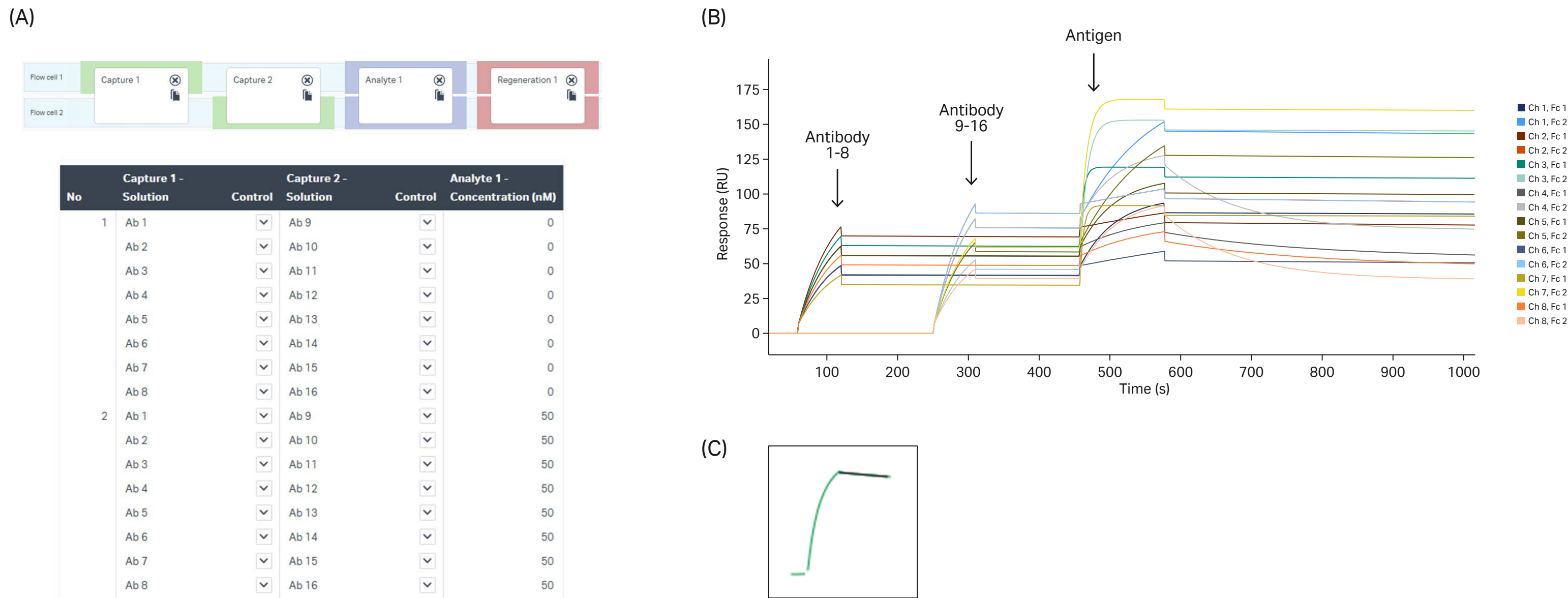


Fig 5. Setup of **Off-rate ranking using 16 flow cells** in the control software (A), sensorgrams showing the two capture steps and analyte injection (B) and sensorgram fit to a 1:1 dissociation model (C).

Tips for antibody screening

- **Select the right assay format from the start**

Choose assay format based on antigen availability and the format of the molecules to be screened. If your goal is to obtain kinetic information, use antibody as ligand. This approach requires more antigen but benefits from simplified surface preparation and reduced avidity effects, increasing the likelihood of reliable kinetic data.

- **Optimize ligand levels to avoid artefacts**

When using antigen as ligand, carefully optimize the immobilization level. Levels that are too low may give weak signals, while levels that are too high can introduce avidity effects for bivalent antibodies.

- **Minimize non-specific binding early**

Evaluate non-specific binding from the sample matrix by injecting blank samples (sample matrix without antibody) and check for binding to both active and reference surfaces.

- **Verify assay specificity before screening**

Test for unwanted binding of antigen to the capture surface in a short pre-experiment where antigen is injected over the capture molecule. Confirming specificity upfront reduces the risk of misleading results during the screen.

- **Use appropriate data collection rates**

Use a data collection rate of 1 Hz. Higher data collection rates increase file sizes without providing additional information.

- **Verify the assay before scaling up**

Test the assay with a small set of samples and controls before running large screening campaigns. This allows you to identify issues early and ensure robust performance in extended runs.

- **Reduce sample dilution when needed**

When using pooled sample positions in microplates, use ***Predip*** to minimize sample dilution.

- **Use blank cycles strategically**

For ***Kinetic screen*** and ***Off-rate ranking***, include blank cycles to enable proper subtraction. For highest accuracy, include one blank per sample. To increase throughput and reduce consumption of captured ligand, a blank cycle can be included at selected intervals and used for all samples. Note that this approach reduces precision since the chosen cycle needs to be representative for the whole screen. For ***Off-rate ranking using 16 flow cells***, one blank per sample is required, as blank subtraction replaces reference subtraction in this format.

General considerations

Surface preparation

Response levels in antibody screening are usually quite high, so it's not necessary to use high ligand levels. Specific considerations for screening approaches using antibody as ligand versus antigen as ligand are provided in the respective sections below (see [Surfaces for antibody capture](#), on page 9 and [Preparing the antigen surface](#), on page 11).

Buffers

HBS-EP+ (HEPES-buffered saline with 3 mM EDTA and 0.05% Surfactant P20, available from Cytiva) or similar is recommended as running buffer for antibody screening. Precise matching of sample and running buffer is neither needed nor practicable for antibody screening work.

Sample preparation

Samples for antibody screening are typically clarified cell culture supernatants or phage display preparations, that can be used without further purification. Samples may be diluted using running buffer.

To verify that the sample matrix does not interact with the sensor surface or the capture molecule, a control experiment is recommended. In the control experiment, only sample matrix (cell culture supernatant or corresponding matrix without antibodies) is injected over both active and reference surfaces. If there is non-specific binding from the sample matrix to the sensor surface, dilute samples with running buffer (typically 1:1). Addition of NSB Reducer at 1 mg/mL can also help reduce non-specific binding.

We also recommended testing for the absence of antigen binding to the capture molecule by injecting antigen over the capture surface. If the antigen binds to the capture molecule, this capture molecule needs to be replaced and the test repeated.

Run recommendations and settings

Screening using antibody as ligand

The first assay format described above (screening using antibody as ligand and antigen as analyte) is the recommended approach for kinetic screening. The steps involved are summarized below.

Step	Action
1	Dock a pre-functionalized sensor chip or couple a capture molecule to the active and reference flow cells of a Series S Sensor Chip CM5.
2	Equilibrate the system by running at least 3 startup cycles. Use buffer and regeneration in the first two cycles. Antibody (of the same species as the antibodies to be tested) and regeneration should be used in the third cycle.
3	Inject sample containing antibodies, such as cell culture supernatants or phage display preparations, over the active surface to capture antibodies from the sample. Crude samples can be injected directly without pre-treatment.
4	Inject antigen, used as the analyte, over the active and reference surfaces. For Kinetic screen , purified antigen of known concentration is needed. Dilute the antigen in running buffer. Approximately 50–100 nM of antigen is usually sufficient.
5	Regenerate the surface to remove captured antibody and bound antigen.
6	For best performance, include a blank cycle for each antibody screened. For Off-rate ranking using 16 flow cells , a blank cycle is needed for each antibody screened since blank subtraction replaces reference subtraction in this assay.
7	Evaluate results.

The experimental setup differs slightly depending on whether results will be evaluated from single report points, or as off-rate or kinetic ranking. Details are given below.

Surfaces for antibody capture

Our ready-to-use kits significantly reduce assay development time, giving consistent preparation of surfaces for capturing mouse and human antibodies or human antibody fragments. Custom capturing molecules can also be coupled to the sensor chip, if required. In general, aim to couple about 5000 RU of the capturing molecule, depending on the expected antibody expression levels and size of the antigen. Using higher levels can lead to non-specific binding from complex samples and does not add value to the experiment.

Sensor chips pre-functionalized with Protein A, Protein G and Protein L are also available from Cytiva. These surfaces have high surface capacities, which can lead to mass transport limitation and re-binding issues for **Kinetic screen** or **Off-rate ranking** assays and are therefore more suitable for report-point based screening. Protein A and Protein G bind selectively to antibodies from different species and subclasses. Protein L binds specifically to antibodies containing kappa light chains, with a broader selectivity than Protein A and Protein G. For samples containing calf serum, use a species-specific capture molecule instead since Protein A and Protein G display specificity to bovine IgG which would lead to unwanted binding of the sample matrix. Ordering information may be found on the *Products* pages at [cytiva.com](https://www.cytiva.com).

Product name	Intended use
Mouse Antibody Capture Kit	Mouse IgG, IgA, and IgM antibodies
Human Antibody Capture Kit	Human IgG antibodies
Human Fab Capture Kit	Human Fab fragments (kappa and lambda)
Sensor Chip Protein A	Antibodies according to the binding profile of Protein A
Sensor Chip Protein G	Antibodies according to the binding profile of Protein G
Sensor Chip Protein L	Antibodies according to the binding profile of Protein L

Recommended capture levels

In general, the antibody concentrations of clones to be screened are unknown. Consequently, precise capture levels are difficult to achieve. If concentration estimates are available, aim for a capture level of approximately 500 RU. Higher capture levels may cause mass transport limitations, resulting in less reliable results.

Conditions for off-rate ranking and kinetic screening

Recommendations for *Off-rate ranking* and *Kinetic screen* are listed in Table 1. Use a predefined run method if one is provided with your Biacore SPR system.

Table 1. Recommended parameters for capture of antibody as ligand and injection of antigen as analyte in *Off-Rate ranking* and *Kinetic screen*

Parameter	Recommended value	Comments
Sample injection (ligand capture)		
Contact time (s)	60 to 180	Long enough to give confidently measurable response levels without compromising throughput.
Flow rate (µL/min)	10	Higher flow rates consume more sample.
Flow path ²	2	
Pooling	Not applicable	
Molecular weight	Not required	
Concentration	Not required	
Antigen injection (analyte)		
Contact time (s)	60 to 120	Use longer contact times if antigen binding is slow.
Dissociation time (s)	180	Use a dissociation time that is long enough to allow estimation of dissociation rates.
Flow rate (µL/min)	No lower than 30	To avoid mass transport limitation.
Injection type	High performance	
Flow path	1 and 2	
Pooling	Optional	Analyte may be pooled to conserve microplate space and reduce antigen consumption.
Molecular weight	Not required	
Concentration	Not required for <i>Off-rate ranking</i>	Use the same concentration in all cycles.
	Molar concentration required for kinetic evaluation	Kinetic screening is performed with a single antigen concentration.

²In Biacore 8 series method *Off-rate ranking using 16 flow cells*, twice as many clones are captured each cycle by injecting clone 1 to 8 in flow cell 1 of channel 1 to 8 followed by clone 9 to16 in flow cell 2 of channel 1 to 8. The remaining steps are the same as above.

Conditions for report point-based screening

Recommendations for report point-based screening, using captured antibody as ligand and antigen as analyte, are listed in Table 2. Use a predefined run method if one is provided with your Biacore system.

Table 2. Recommended parameters for capture of antibody as ligand and injection of antigen as analyte in report point-based screening

Parameter	Recommended value	Comments
Sample injection (ligand capture)		
Contact time (s)	60 to 180	Long enough to give confidently measurable response levels without compromising throughput.
Flow rate (µL/min)	10	Higher flow rates consume more sample.
Flow path	2	
Pooling	Not applicable	
Molecular weight	Not required	
Concentration	Not required	
Antigen injection (analyte)		
Contact time (s)	60	Use longer contact times if antigen binding is slow.
Dissociation time (s)	60 to 120	Include a dissociation time to provide a rough indication of antigen binding stability.
Flow rate (µL/min)	Not lower than 30	To avoid mass transport limitations that can obscure differences in ligand levels.
Injection type	Low sample consumption	Saves antigen when no kinetics is required.
Flow path	1 and 2	
Pooling	Optional	Analyte may be pooled to conserve microplate space and reduce antigen consumption.
Molecular weight	Not required	
Concentration	Not required	Use the same concentration in all cycles.

Blank cycles

Include blank cycles for **Off-rate ranking** and **Kinetic screen**. Blank cycles consist of antibody capture followed by injection of buffer instead of antigen. Ideally, there should be a blank cycle for each sample. Throughput can be improved by using a general blank cycle at intervals (for example, every 5 or 10 sample cycles), using a representative clone for the antibody capture. This approach requires that the antibody capture cycles are similar throughout the assay with respect to parameters such as capture level and drift. However, **Off-rate ranking using 16 flow cells** requires a blank cycle for each sample since no reference subtraction is used in this assay.

Blank cycles are not necessary for report point-based screening.

Screening using antigen as ligand

Antibody screening with antigen as ligand is performed with single injections of each antibody clone over antigen on the surface. The antigen may be covalently coupled or captured. A typical antibody screening experiment with coupled antigen includes the following steps:

Step	Action
1	Couple or capture the antigen on the sensor surface. Assay development work may be required to establish suitable coupling conditions, ligand level, and regeneration conditions.
2	Run at least three startup cycles using buffer and regeneration to equilibrate the system.
3	Inject samples containing antibodies, such as cell culture supernatants or phage display preparations, over the active and the reference surfaces. Crude samples can be injected directly, without pre-treatment.
4	Regenerate the surface to remove antigen and/or bound antibody.
5	Evaluate results.

Preparing the antigen surface

If the antigen is to be covalently coupled to the surface, assay development work will most likely be required to establish coupling and regeneration conditions. It is important that coupling and regeneration do not interfere with antibody binding. Scouting strategies for coupling and regeneration conditions are described in *Biacore sensor surface handbook*.

Antigens that carry a suitable tag can be captured on the sensor surface. In some cases, optimization of capture level may be required since a capture level that's too high can lead to baseline drift. For most tags, regeneration removes both bound antibody and antigen from the surface, so that fresh antigen is captured for each cycle. However, when biotinylated antigens are captured on streptavidin-coated surfaces, the strong biotin–streptavidin interaction allows bound antibodies to be removed during regeneration without disrupting the captured antigen on the sensor surface. With this approach, antigen is captured once at the beginning of the screen. Since the same antigen is reused, a positive control can be included to verify maintained antigen activity throughout the assay.

Ready-to-use kits and pre-functionalized sensor surfaces for capturing tagged antigens are on our *Products* pages at [cytiva.com](https://www.cytiva.com).

Product name	Intended use
Biotin CAPture Kit	Reversible capture of biotinylated antigens
Sensor Chip SA	Permanent capture of biotinylated antigens
His Capture Kit	Histidine-tagged antigens
Sensor Chip NTA	Histidine-tagged antigens

Conditions for report point-based screening

Recommended injection conditions for antibody samples run as analytes over coupled or captured antigen are listed in Table 3.

Table 3. Recommended parameters for antibody samples run as analytes in report point-based screening

Parameter	Recommended value	Comments
Contact time (s)	60 to 180	Long enough for confidently measurable response levels without compromising throughput.
Dissociation time (s)	60	A long dissociation time is not needed since off-rate and kinetics are not evaluated.
Flow rate (μL/min)	10	Higher flow rates consume more sample.
Injection type	Low sample consumption	Saves sample when no kinetics is required.
Flow path	1 and 2	

Blank cycles

Blank cycles are not necessary for report point-based screening.

Evaluation tools and options

Antibody screens can be evaluated with a range of options to display screening results, according to requirements and to some extent personal preferences. Several of the options described in this section are implemented automatically by predefined evaluation methods in Biacore Insight evaluation software.

Table 4 lists the information provided by different items in Biacore Insight evaluation software.

Table 4. Information provided by different evaluation items in Biacore Insight evaluation software

Item	Antibody as ligand	Antigen as ligand
Sensorgram	Quality control Antibody capture and antigen binding	Quality control Antibody binding
Kinetic screen using 1:1 binding model	Antigen binding kinetics (k_a and k_d)	Not recommended
Off-rate raking (Kinetic screen using 1:1 dissociation model)	Antigen binding stability (k_d)	Not recommended
Plot of capture_level against cycle	Relative expression level	Not generally applicable. For screening using capture on CAP, NTA or anti-His surfaces, the capture level may provide a check on surface performance throughout the assay.
Plot of stability_early against cycle	Relative expression level and antigen binding capacity	Relative expression level/ and antigen binding capacity
Plot of stability_early against capture_level	Antigen binding capacity adjusted for expression level	Not applicable
Plot of stability_early against stability_late	Antigen binding stability together with relative expression level and antigen binding capacity	Antigen binding stability together with relative expression level and antigen binding capacity

Note: The report points *stability early* and *stability late* refer to the analyte injection (antigen when antibody is used as ligand and vice versa).

Sensorgrams

Sensorgrams can be aligned to zero at baseline before capture injection or before the analyte injection, to provide different perspectives on capture and binding behavior (Fig 6, 7). By visualizing the analyte binding, non-binding clones can be identified and excluded from further analysis by right-clicking on the sensorgram and selecting "exclude global". This procedure simplifies the interpretation of downstream analyses.

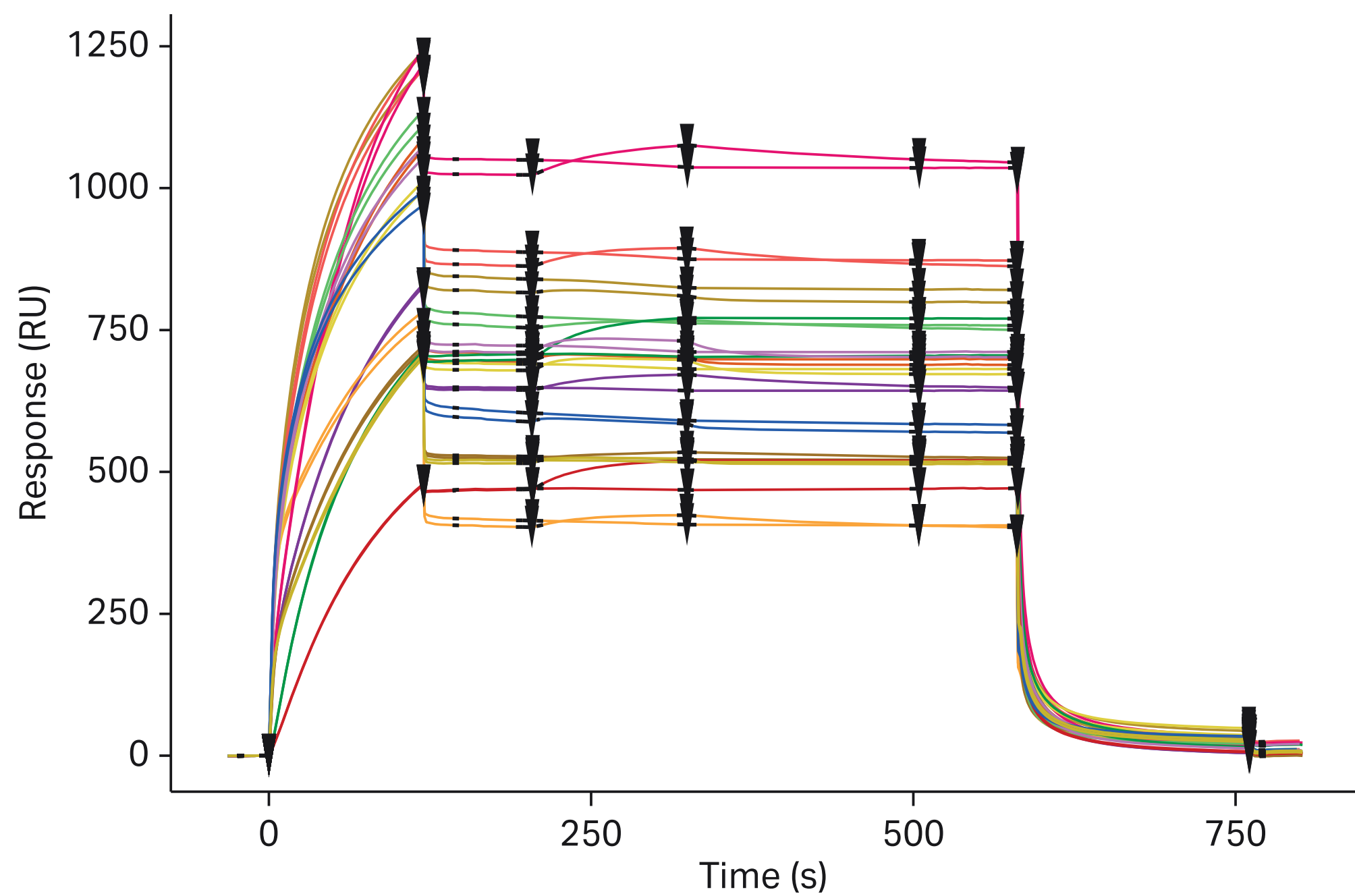


Fig 6. Sensorgrams aligned before the capture injection from screening using captured antibodies as ligands and antigen as analyte.

Sensorgrams can also identify disturbances in a cycle that might not be visible in report point plots. If a disturbance affects kinetic fitting or report points, the cycle should be excluded from the evaluation. Sensorgrams with deviating binding profile can be tagged using curve markers as a reminder for subsequent analysis.

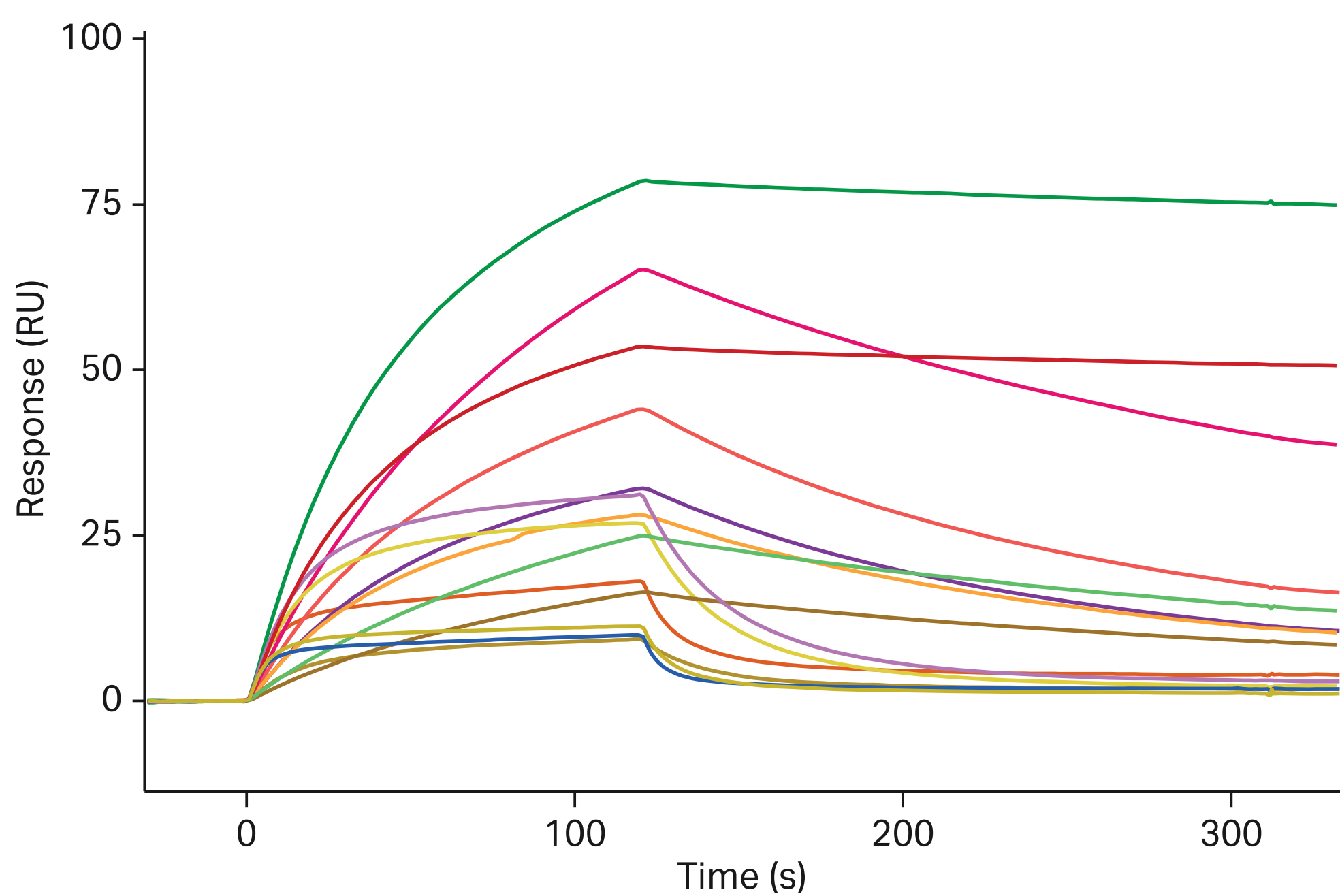


Fig 7. Sensorgram aligned before analyte injection in kinetic screen using antibody as ligand.

Kinetic screen

A **Kinetic screen** is evaluated by fitting the association and dissociation phases of single-concentration antigen injections to a 1:1 binding model. Reference and blank subtraction should be performed before the data is evaluated. In Biacore 8 series, a **Kinetic screen** can only be performed in 8 flow cell mode since flow cell 1 is reserved for reference subtraction. A molar value for antigen concentration is required for data fitting. Since the same antigen concentration is used for all cycles, an approximate value can be used to obtain relative kinetic parameters if the exact value is not known. Inaccuracy in the molar concentration will be reflected in the reported association rate constants, while dissociation rate constants will be unaffected since k_d is not dependent on antigen concentration.

A plot of association rate constant k_a against dissociation rate constant k_d , often called an *on-off rate chart*, is valuable in visualizing results of a **Kinetic screen**. In this plot, antibody clones with identical affinity lie along the same diagonal. Clones that have different rate constants, but identical affinity are distributed along this diagonal. The affinity constant is equal to the ratio of the rate constants for a 1:1 interaction ($K_D = k_d/k_a$).

Biacore systems that support kinetic screening provide graphical and numerical overviews of the results (Fig 8, Table 5).

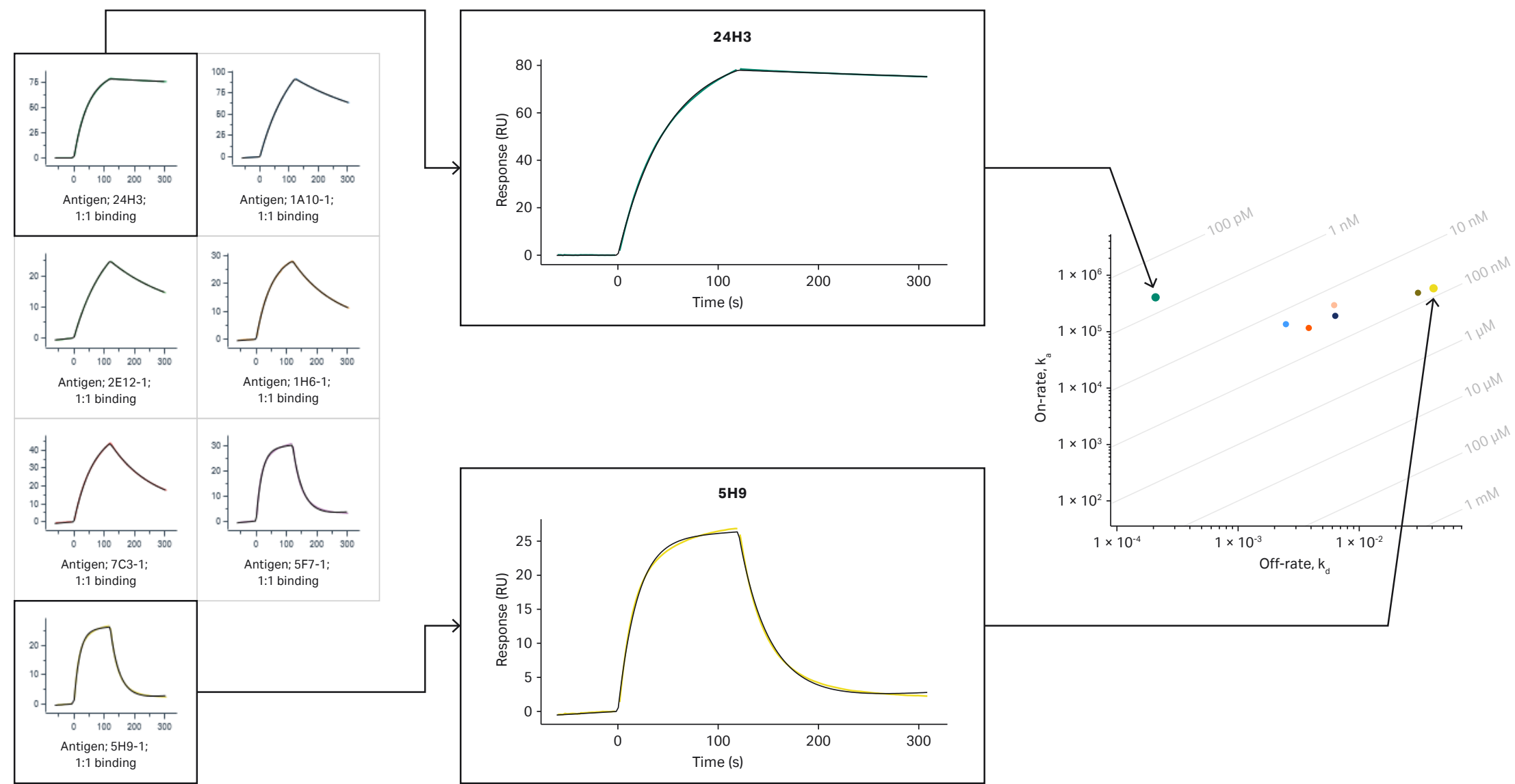


Fig 8. Thumbnails showing a 1:1 binding model fit to a **Kinetic screen** dataset. Larger sensorgrams display the fit of the 1:1 binding model to the clones with slowest and fastest off-rates, respectively.

Table 5. A 1:1 binding model fit to a kinetic screening dataset using the **Kinetic screen** evaluation method

Capture 1 solution	Analyte 1 concentration (nM)	1:1 binding k_a (1/Ms)	k_d (1/s)	R_{max} (RU)	K_D (M)	U-value
24H3	50	4.1×10^5	2.1×10^{-4}	86	5.1×10^{-10}	2
1A10-1	50	1.4×10^5	2.5×10^{-3}	181	1.8×10^{-8}	1
2E12-1	50	1.2×10^5	3.8×10^{-3}	58	3.3×10^{-8}	1
1H6-1	50	3.0×10^5	6.2×10^{-3}	42	2.1×10^{-8}	1
7C3-1	50	1.9×10^5	6.3×10^{-3}	83	3.3×10^{-8}	1
5F7-1	50	4.9×10^5	3.1×10^{-2}	65	6.3×10^{-8}	1
5H9-1	50	5.9×10^5	4.1×10^{-2}	61	7.0×10^{-8}	1

Off-rate ranking

Off-rate ranking is evaluated by fitting the dissociation phase of single-concentration antigen injections to a 1:1 dissociation model. Reference and blank subtraction should be performed before the data is evaluated. The model describes an exponential process that is independent of starting concentration. As a result, antigen concentration is not required as input.

In Biacore 8 series systems, **Off-rate ranking** can also be performed without reference subtraction. The reference flow cell is, in this case, used for ligand capture, and the run method contains two capture steps, one in flow cell 1 (fc 1) and another in flow cell 2 (fc 2). A blank cycle (preceding the antigen cycle) is included for each sample. The results are analyzed using the

Off-rate ranking using 16 flow cells evaluation method where fc 1 and fc 2 are set to active. Each sensorgram is blank subtracted (but not reference subtracted) before fitting to a 1:1 dissociation model. Blank subtraction removes capture drift but injection related disturbances such as bulk shifts might remain. This is why report point analysis or kinetic fitting for a 16-flow cell run is limited to the dissociation phase which is less affected by injection related disturbances.

Biacore SPR systems that support **Off-rate ranking** for screening purposes provide both graphical and numerical overviews of the results (Fig 9 and Table 6).

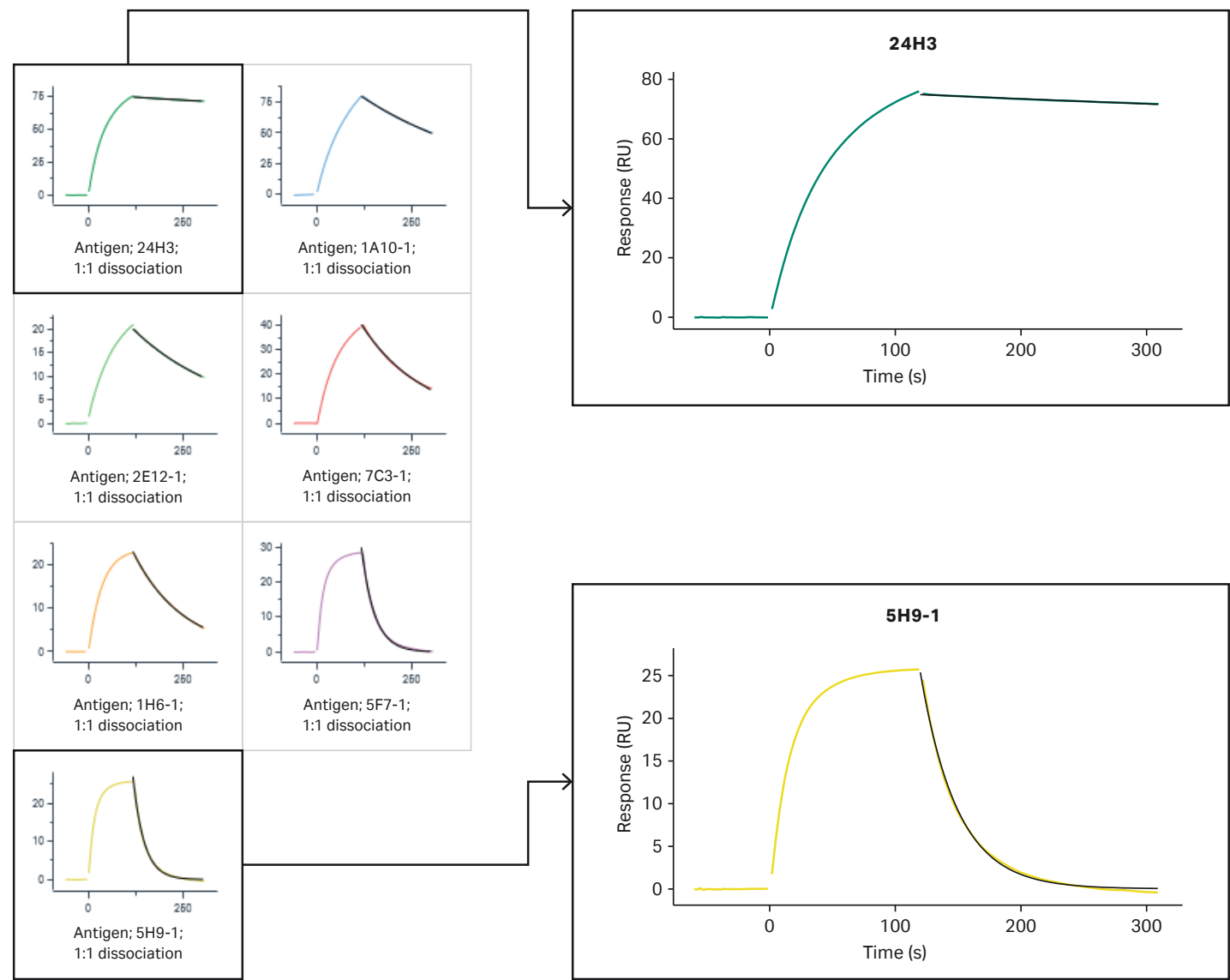


Fig 9. Thumbnails showing a 1:1 dissociation model fit to a 16-flow cell antibody screening dataset using the **Off-rate ranking using 16 flow cells** evaluation method. Larger sensorgrams display the fit of the 1:1 dissociation model to the clones with slowest and fastest off-rates, respectively.

Table 6. A 1:1 dissociation model fit to 16-fc antibody screening dataset using the **Off-rate ranking using 16 flow cells** evaluation method

Channel	Flow cell	Capture 1 solution	Capture 2 solution	Analyte 1 solution	1:1 dissociation k_d (1/s)	Quality kinetics χ^2 (RU ²)
6	2		24H3	Antigen	2.4×10^{-4}	6.2×10^{-3}
2	2		1A10-1	Antigen	2.6×10^{-3}	1.4×10^{-1}
6	1	2E12-1		Antigen	3.9×10^{-3}	4.7×10^{-3}
3	1	7C3-1		Antigen	5.9×10^{-3}	1.4×10^{-1}
4	1	1H6-1		Antigen	7.7×10^{-3}	3.4×10^{-3}
7	1	5F7-1		Antigen	2.9×10^{-2}	1.4×10^{-1}
5	1	5H9-1		Antigen	3.4×10^{-2}	6.6×10^{-2}

Report point-based screening

The following plot items are used in report point-based screening. However, they can also be used as complementary tools in **Kinetic screen** or **Off-rate ranking** evaluations.

Capture_level against cycle

A plot of **capture_level** against cycle number gives a good indication of the relative expression levels of different clones, on the assumption that non-specific binding of material to the surface can be ignored (Fig 10A). This assumption can be tested by injecting blank samples with same sample matrix composition as the tested clones over the surface as described previously.

Clones that do not produce significant amounts of antibody give capture levels close to background. These clones can be excluded from further evaluation if desired.

Stability_early against cycle

The report point **stability_early** is placed immediately after the antigen injection.

A plot of **stability_early** against cycle number provides a combined indication of 2 parameters (Fig 10B):

- Relative expression level: clones that produce low levels of antibody will show correspondingly low levels of antigen binding
- Antigen binding properties: antibodies that bind antigen slowly or weakly will show low levels of antigen binding even if the expression levels are high

The plot of **stability_early** against cycle number does not distinguish between these causes. However, clones can often be excluded from further work based on low antigen responses, regardless of the cause.

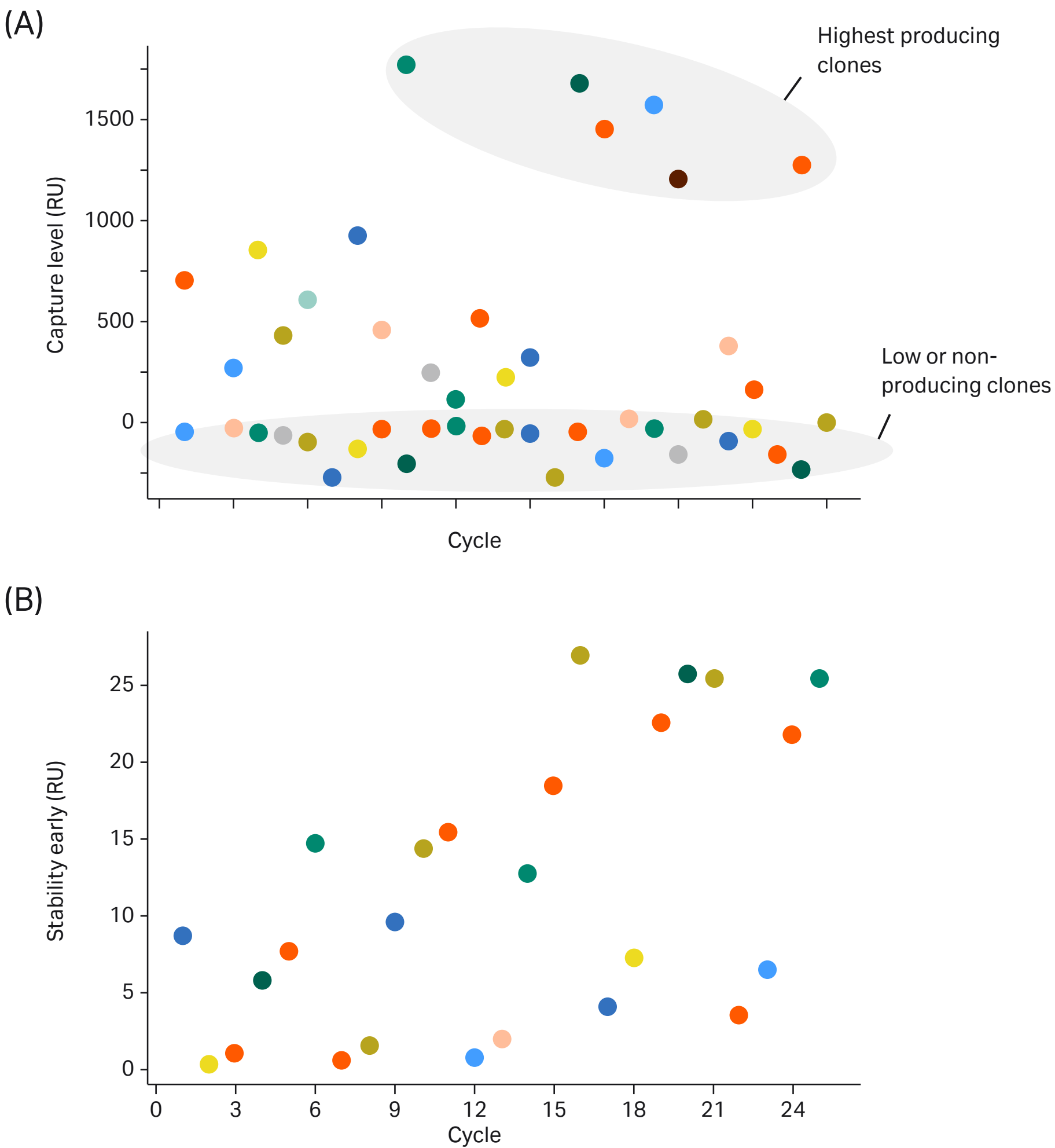


Fig 10. **Capture_level** vs cycle plot showing the relative expression levels of the different clones (A). **Stability_early** vs cycle plot showing the combined contribution of relative expression level and binding affinity, which cannot be resolved independently in this plot (B).

Stability_early against capture_level

A plot of **capture_level** against **stability_early** can distinguish between clones that express low levels of antibody and antibodies that bind antigen weakly (Fig 11). Points for low expression levels lie close to the **stability_early** axis, while weak binders lie close to the **capture_level** axis.

The same information can be obtained by adjusting the antigen response for the capture level in systems that support this function.

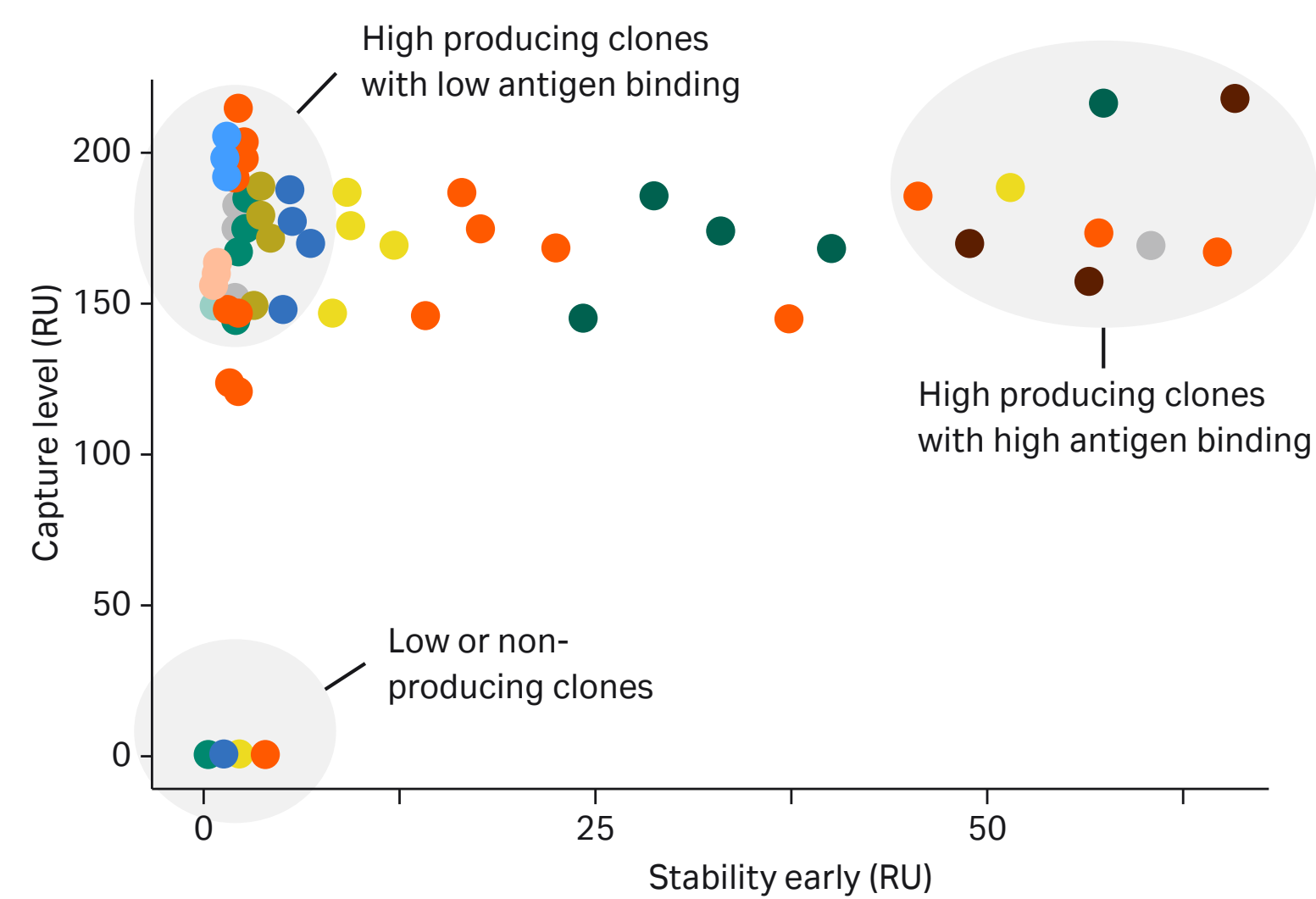


Fig 11. **Capture_level** vs **stability_early** plots can distinguish the clone’s relative expression level from binding affinity and resolve clones with weak antibody expression from clones expressing antibodies with weak antigen binding.

Stability_early against stability_late

The report point **stability_late** is placed just before the end of the antigen dissociation. A plot of **stability_early** against **stability_late** gives an indication of antigen binding stability (Fig 12). However, more detailed information can be obtained through **Off-rate ranking** and **Kinetic screen**.

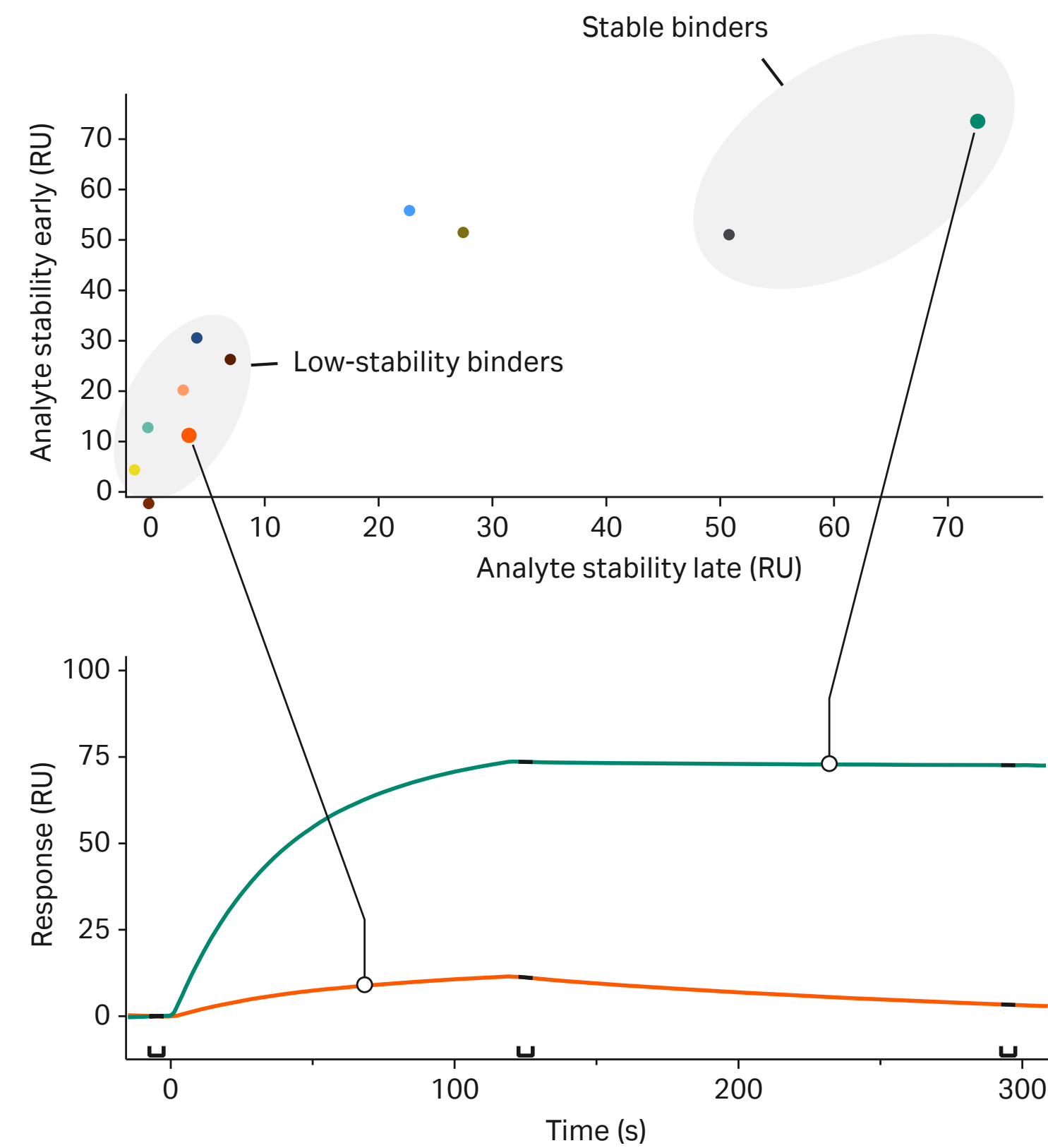


Fig 12. A **stability_early** (SE) vs **stability_late** (SL) plot can be used to illustrate the dissociation rate of an antigen from a captured antibody. Stable binders with slow dissociation exhibit high SE and SL. Low-stability binders may have either high or low SE with low SL reflecting rapid dissociation.



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