Biacore application guides

Antibody screening with Biacore systems

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In the search for therapeutic or analytical antibodies the selection of appropriate kinetics/affinity, specificity and biophysical properties is essential. Antibody screening aims to identify cell clones that produce antibodies appropriate for the purpose, by challenging the antibodies with known antigen. Sample matrices are often complex, e.g. hybridoma culture supernatants or phage display preparations. Obtaining kinetic information early in the screening process is often a significant advantage. Information that is generally sought includes:

- Specificity — which clones produce antibodies with the desired specificity?
- Binding characteristics — which clones produce antibodies with suitable kinetic and/or affinity properties?
- Expression level — which clones produce sufficient amounts of antibodies?

Screening can be performed on full size antibodies or parts thereof, such as Fab fragments or scFvs. This *Application guide* deals with screening of complete monoclonal IgG antibodies. Assay conditions and prerequisites might differ for other antibody types and fragments.

Assay format

Antibody screening can be run using two different assay formats:

Format	Description
Antibody as ligand Antigen as analyte	Samples to be screened are injected over a generic antibody-capturing surface. Antigen is then injected as analyte[1]. The surface is regenerated by removing captured antibody and bound antigen.
Antigen as ligand Antibody as analyte	Antigen is immobilized or captured on the surface. Samples to be screened are then injected as analyte. The surface is regenerated by removing the bound antibody. (If reversible antigen capture is used, the surface may be regenerated by removing captured antigen and bound antibody).

[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 thiscase the captured ligand.

Capturing the antibody as ligand is generally more straightforward with respect to surface preparation and assay development, but requires injection of antigen for each cycle in the screen. Using the antigen as ligand can involve additional work to establish immobilization and regeneration conditions.

Screening using antigen as ligand is only recommended for assessment of specificity and/or expression level, for several reasons:

- Antibody concentrations are usually not known, and are required for kinetic screening
- Complete antibodies are usually bivalent, making evaluation of off-rate ranking and kinetic screening more difficult

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

Screening types

Information from antibody screens using Biacore systems may be obtained at different levels as summarized below.

Screening type	Description
Report point-based screening	Provides information on specificity and expression level from single point binding level measurements.
Off-rate ranking	Provides comparative information on the dissociation rate of the antibody-antigen complex.
Kinetic screening	Provides information on both the association and dissociation rates of antibody-antigen binding.

Frequently, report point-based screening is used as an initial screen to eliminate non-producing clones. Off-rate ranking and kinetic screening are then used for more detailed characterization of the clones that produce potentially interesting antibodies.

Tips for antibody screening

- Choose the assay format depending on your needs and preferences. Choose antibody as ligand if your aim is to obtain kinetic information.
- When using antigen as ligand, estimate how much you need to immobilize in order to obtain high enough responses from the injected antibody
- Check for non-specific binding from the sample matrix by injecting blank samples (sample matrix without antibody) and check for binding to both the active and reference surfaces
- A data collection rate of 1 Hz is usually sufficient. Using higher data collection rates increases file sizes without providing additional information.
- Establish that the assay is suitable for purpose using a few samples and controls before you start extended runs with many samples
- If you are using pooled sample positions in the microplate, use **Predip** with the injection to minimize sample dilution
- For off-rate ranking and kinetic screening, consider the use of blank cycles. For best performance include one blank cycle per sample. To increase throughput and reduce antigen consumption a blank cycle can be included at selected intervals and used for all samples. Note that this approach is generally less precise.

General considerations

Surface preparation

Response levels in antibody screening are usually quite high, and it is not necessary to use high ligand levels. Specific considerations for screening approaches with antibody as ligand and antigen as ligand are given in the respective sections below (see *Surfaces for antibody capture*, on page 5 and *Preparing the antigen surface*, on page 8 respectively).

Buffers

HBS-EP+ (HEPES-buffered saline with 0.3 mM EDTA and 0.05% Surfactant P20, available from Cytiva) or similar is recommended as running buffer for antibody screening. Samples may be diluted if required using the same buffer. Precise matching of sample and running buffer is neither needed nor practicable for antibody screening work, since the report points used are placed after the sample injections.

Sample preparation

Samples for antibody screening are typically clarified material such as hybridoma culture supernatants or phage display preparations, used without further purification. To verify that there is no binding of the sample matrix to the surface a control experiment is recommended in which only the sample matrix (cell culture supernatant or corresponding matrix) is injected over the sensor surface.

If there is non-specific binding from the sample matrix this can be reduced by diluting the samples with running buffer (typically 1:1). Addition of NSB Reducer (available from Cytiva) at 1 mg/mL can also help to reduce non-specific binding.

Screening using antibody as ligand

The general approach to screening with antibody as captured ligand is summarized below.

Step	Action
1	Dock a pre-immobilized sensor chip or immobilize the capturing molecule.
2	Run at least 3 startup cycles using buffer and regeneration to equilibrate the system.
3	Inject sample containing antibodies (for example, hybridoma 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 as analyte over both the active and reference surfaces.
5	Evaluate the results.

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

Surfaces for antibody capture

Ready-to-use kits for preparing surfaces for capture of mouse and human antibodies and human antibody fragments are available from Cytiva (see the table below). Custom capturing molecules may also be immobilized on the sensor chip if required. In general, aim to immobilize about 5000 RU or less of the capturing molecule, depending on the expected antibody expression levels and the size of the antigen. Using higher levels can lead to non-specific binding from complex samples.

Sensor chips preimmobilized with Protein A (MabSelect SuRe™), Protein G and Protein L are also available from Cytiva. 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. Ordering information may be found on the Products pages at www.cytiva.com/biacore.

Product name	Intended for
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

Conditions for report point-based screening

Recommendations for report point based screening are listed below. Use the predefined screening method if one is provided with your Biacore system.

Parameter	Recommended value	Comments
Sample injection (ligand capture)		
Flow rate	10 µL/min	Higher flow rates consume more sample.
Contact time	60 to 180 s	Long enough to give confidently measurable response levels without compromising throughput.
Pooling	Not applicable	
Molecular weight	Not required	
Concentration	Not required	
Antigen injection (analyte)		
Flow rate	Not lower than 30 µL/min	Avoid mass transport limitations that can obscure differences in ligand levels.
Injection type	Low sample consumption	
Contact time	60 s	Use longer contact times if antigen binding is slow.
Dissociation time	120 s	Include a dissociation time to provide a rough indication of antigen binding stability.
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.

Conditions for off-rate ranking and kinetic screening

Recommendations for off-rate ranking and kinetic screening are listed below.

Parameter	Recommended value	Comments
Sample injection (ligand capture)		
Flow rate	10 µL/min	Higher flow rates consume more sample.
Contact time	60 to 180 s	Long enough to give confidently measurable response levels without compromising throughput.
Pooling	Not applicable	
Molecular weight	Not required	
Concentration	Not required	
Antigen injection (analyte)		
Flow rate	Not lower than 30 µL/min	Avoid mass transport limitations.
Injection type	High performance	
Contact time	60 to 120 s	Use longer contact times if antigen binding is slow.
Dissociation time	180 s	Use a dissociation time that is long enough to allow estimation of dissociation rates.
Pooling	Optional	Analyte may be pooled to conserve microplate space and reduce antigen wastage.
Molecular weight	Not required	
Concentration	Not required for off-rate ranking	Use the same concentration in all cycles.
	Molar concentration required for kinetic evaluation	Kinetic screening is performed with a single antigen concentration.

Blank cycles

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

Include blank cycles for off-rate ranking and kinetics screening. 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 fairly similar throughout the assay with respect to parameters such as non-specific binding and drift.

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 immobilized or captured. No control samples are included. A typical antibody screening experiment with immobilized antigen includes the following steps:

Step	Action
1	Immobilize or capture the antigen on the sensor surface. Assay development work may be necessary to establish suitable immobilization conditions.
2	Run at least 3 startup cycles using buffer and regeneration to equilibrate the system.
3	Inject samples containing antibodies (for example, hybridoma culture supernatants or phage display preparations) over both the active and reference surfaces. Crude samples can be injected directly, without pre-treatment.
4	Evaluate the results.

Preparing the antigen surface

If the antigen is to be immobilized covalently on the surface, assay development work will probably be required to establish immobilization chemistry and conditions as well as regeneration conditions. It is important that the immobilization and regeneration do not interfere with antibody binding. Scouting strategies for immobilization and regeneration conditions are described in the *Biacore Sensor Surface Handbook*.

Antigens that carry a suitable tag can be captured on the sensor surface. For most tags, regeneration removes both bound antibody and antigen from the surface, so that fresh antigen is captured for each cycle. Capture of biotinylated antigens on streptavidin surfaces however involves such a high affinity interaction that regeneration can remove the bound antibody but leave the antigen intact on the surface. With this approach, antigen is captured once at the beginning of the screen.

Ready-to-use kits and pre-immobilized sensor surfaces for capturing tagged antigens are available from Cytiva. Ordering information may be found on the *Products* pages at www.cytiva.com/biacore.

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

Conditions for screening

Recommended injection conditions for antibody samples are listed below.

Parameter	Recommended value	Comments
Flow rate	10 µL/min	Higher flow rates consume more sample.
Contact time	60 to 180 s	Long enough to give confidently measurable response levels without compromising throughput.
Dissociation time	0	A dissociation time is not needed since off-rate evaluation is not used.

Evaluation tools and options

Antibody screens can be evaluated with a range of options for displaying the 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 the system software.

The table below lists the information provided by commonly used options.

Display option	Antibody as ligand	Antigen as ligand
Sensorgram display	Quality control	Quality control
Plot of ***capture_level*** against cycle	Expression level	Not generally applicable. However, 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	Expression level/antigen binding capacity	Expression level/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 expression level/antigen binding capacity	Antigen binding stability together with expression level/antigen binding capacity
Off-rate ranking	Antigen binding stability	*Not recommended*
Kinetic screening	Antigen binding kinetics	*Not recommended*

Note: The report points stability_early and stability_late refer to the analyte injection (antigen when antibody is used as ligand and vice versa).

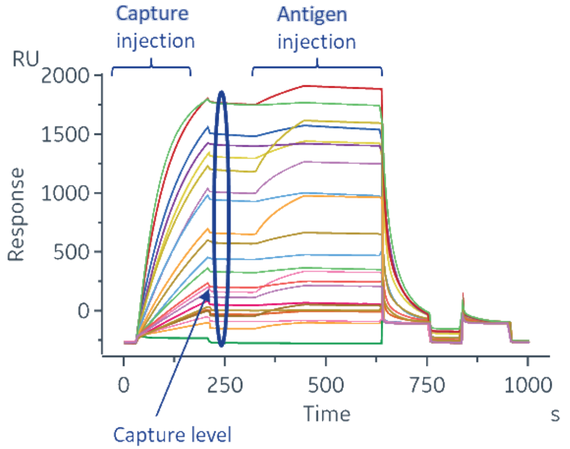
Sensorgrams

Sensorgram display can identify any disturbed cycles. Disturbed cycles should be excluded from the evaluation. Disturbances may not be apparent in report point plots.

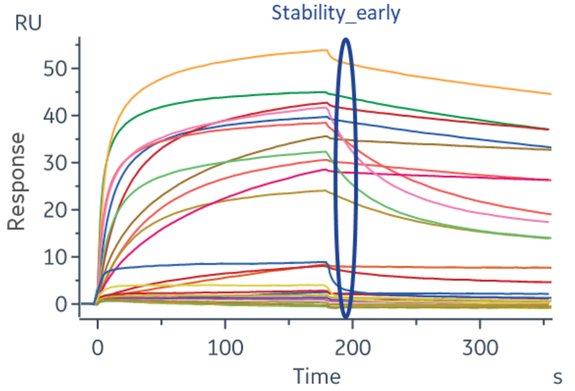
Sensorgrams can be aligned to zero at the baseline before the capture injection or before the antigen injection, to provide different perspectives on the capture and binding behavior.

Examples:

Sensorgrams from screening with antibody as ligand



Sensorgrams from screening with antigen as ligand



Capture_level against cycle

A plot of ***capture_level*** against cycle number gives a good indication of the relative expression levels of the different clones, on the assumption that non-specific binding of material to the surface can be ignored.

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

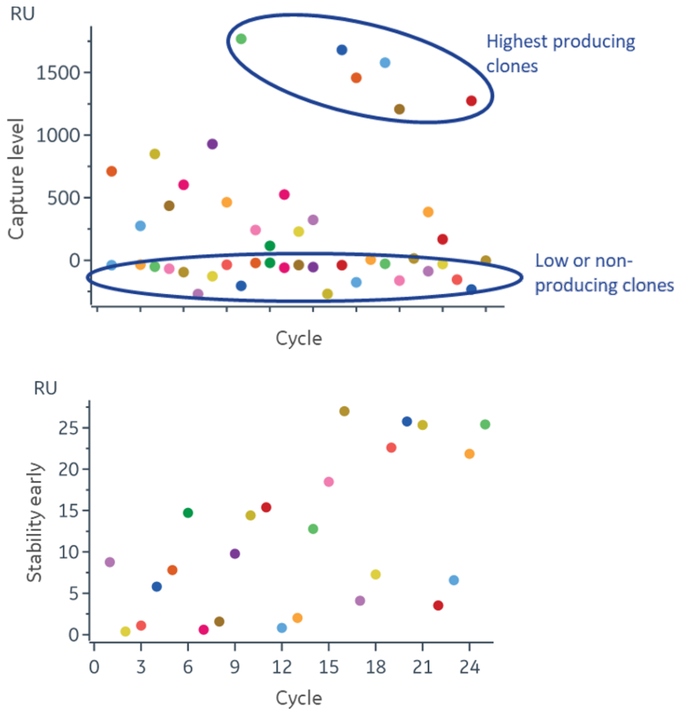
The report point ***stability_early*** is placed immediately after the antigen injection.

A plot of ***stability_early*** against cycle number provides a combined indication of 2 parameters:

- 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 on the basis of low levels of antigen binding, regardless of the cause.

Examples:



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. Points for low expression levels lie close to the ***stability_early*** axis, while weak binders lie close to the ***capture_level*** axis.

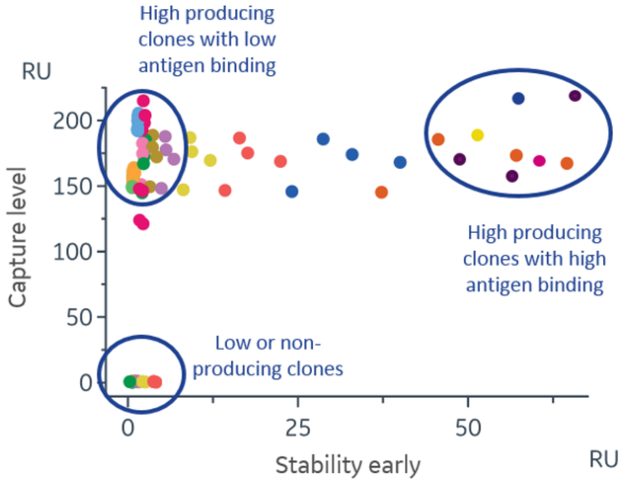
The same information may be obtained by adjusting the antigen response for the capture level in systems that support this function.

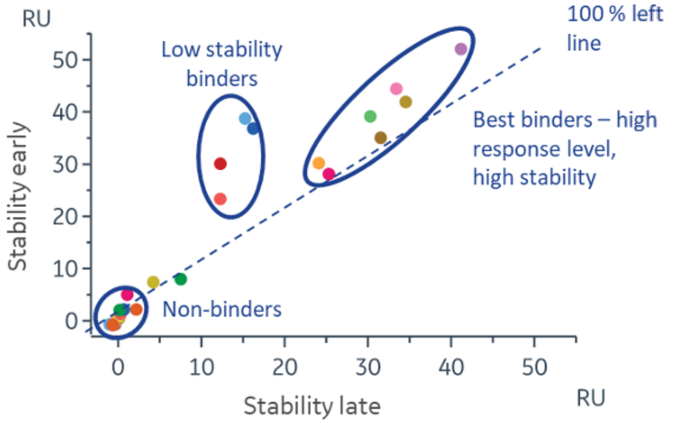
Stability_early against stability_late

The report point ***stability_late*** is placed just before the end of the dissociation time after the antigen injection.

A plot of ***stability_early*** against ***stability_late*** gives an indication of antigen binding stability, as illustrated to the right. More detailed information can be obtained from off-rate ranking and kinetic screening.

Examples:



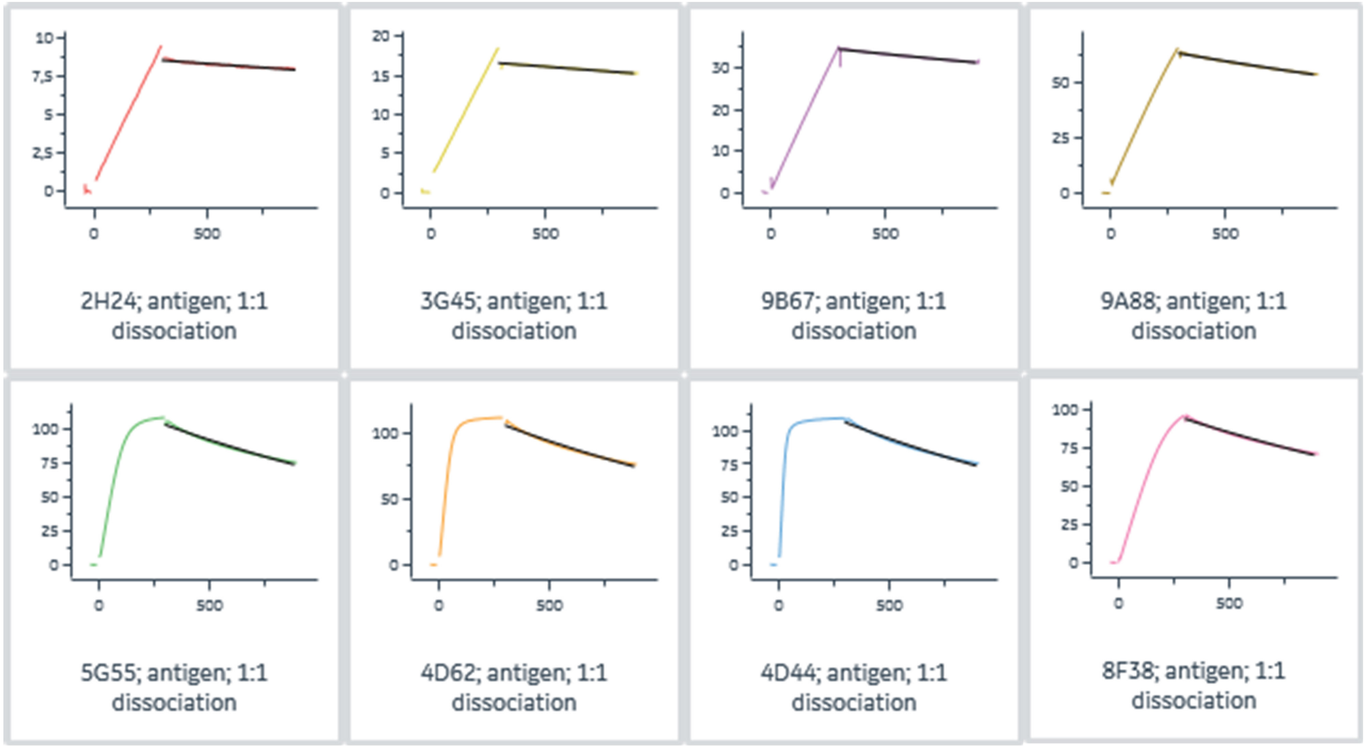


Off-rate ranking

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

Biacore systems that support off-rate ranking for screening purposes provide both graphical and numerical overview of the results.

Example:

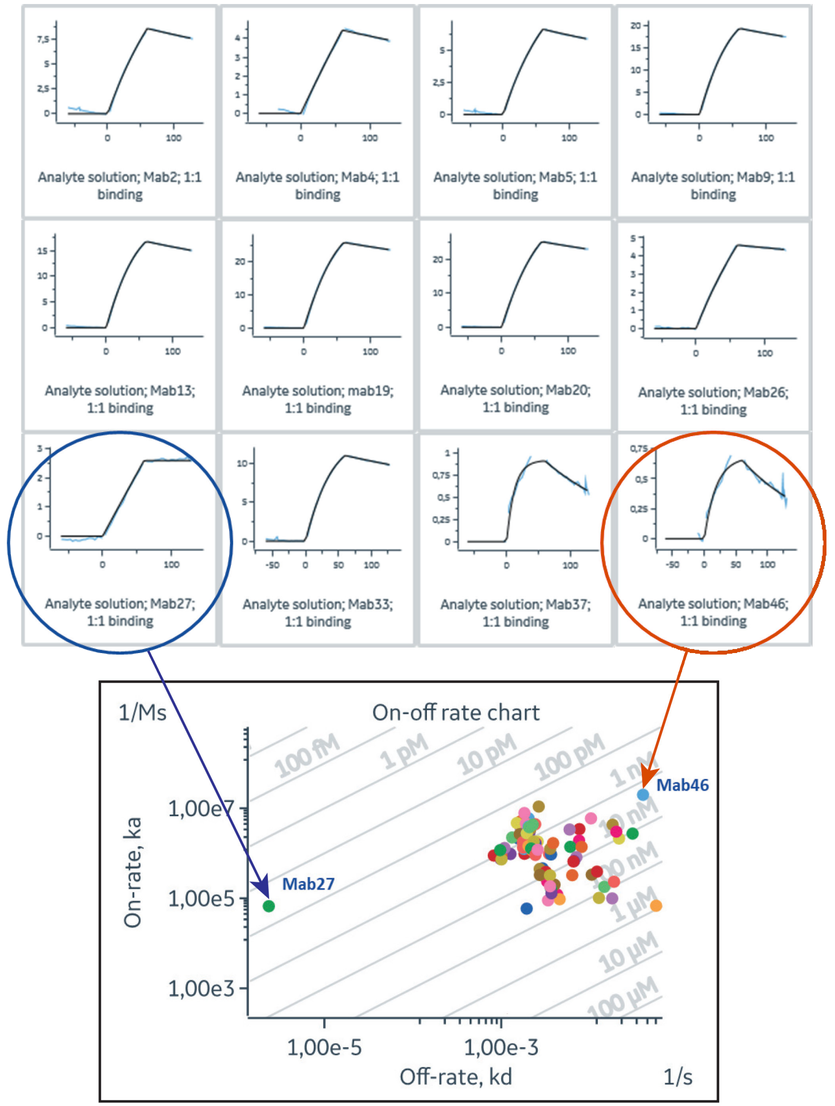


Kinetic screening

Kinetic screening is evaluated by fitting the association and dissociation phases of single-concentration antigen injections to a 1:1 binding model. Blank subtraction should be performed before the data is evaluated. A molar value for antigen concentration is required for the fitting. However, 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.

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 the results of a kinetics screen. In this plot, antibody clones with the same affinity lie on the same diagonal. Clones with different rate constants but the same affinity are distributed along the diagonal. (The affinity constant is equal to the ratio of the rate constants for a 1:1 interaction.)

Example, showing thumbnails and on-off rate chart:



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