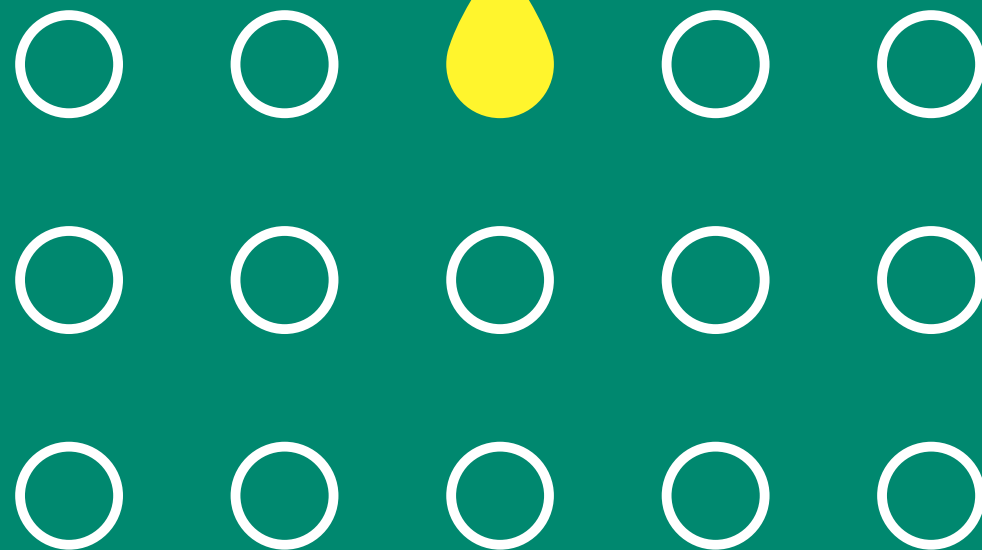


Cytiva Webinar

まもなく開始します。
もうしばらくお待ちください。

※開始時刻から30秒ほど遅れての配信となります。

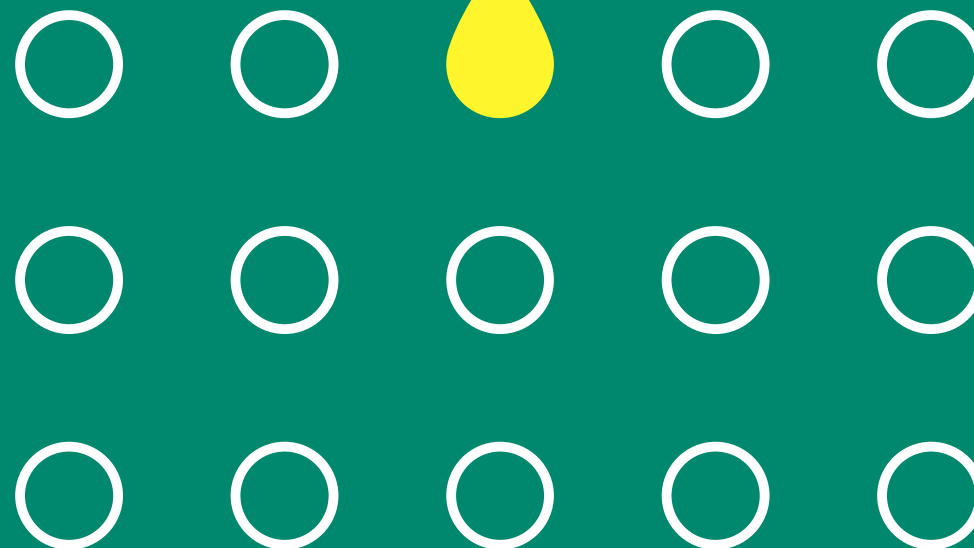


音声につきまして

- 視聴者の皆様の音声は講師、他の参加者には届きません。

ご質問につきまして

- 画面右上のはてなマークをクリックして現れる画面に質問内容を入力してください。
- 講演後まとめて講師より回答いたします。
- 入力いただいたご質問内容、質問者のお名前は、主催者にのみ公開されます。





イチからはじめる Biacoreでの 抗体スクリーニング

Prepared for Masami Koinuma

August 25, 2020

Agenda

1. Biacoreで抗体スクリーニングするメリットは？
2. Assay format と解析
3. 具体的なアプリケーション紹介
4. まとめ

1

**Biacoreで抗体スクリーニング
するメリットは？**

どんな視点で抗体をスクリーニングしますか？

標的に対する特異性（選択性）

どのクローンが期待する特異性を持つ抗体を産生するか？

- エピトープマッピング...部位特異的な阻害や活性化も可能に(治)。サンドイッチ系の開発にも(診)。
- 交叉性...抗原に類似したエピトープを持つタンパク質との非特異的結合を避ける。

標的に対する結合特性

どのクローンが期待するkineticsあるいはaffinityを持つ抗体を産生するか？

- Kinetics/ Affinity解析...用途に合った抗体の選別。
- EC50やIC50...アゴニストとしてのEC50やアンタゴニストとしてのIC50の算出。直接結合と相関性を得る。
- 細胞を用いたスクリーニング...in vivoでの評価として。直接結合と相関性を得る。

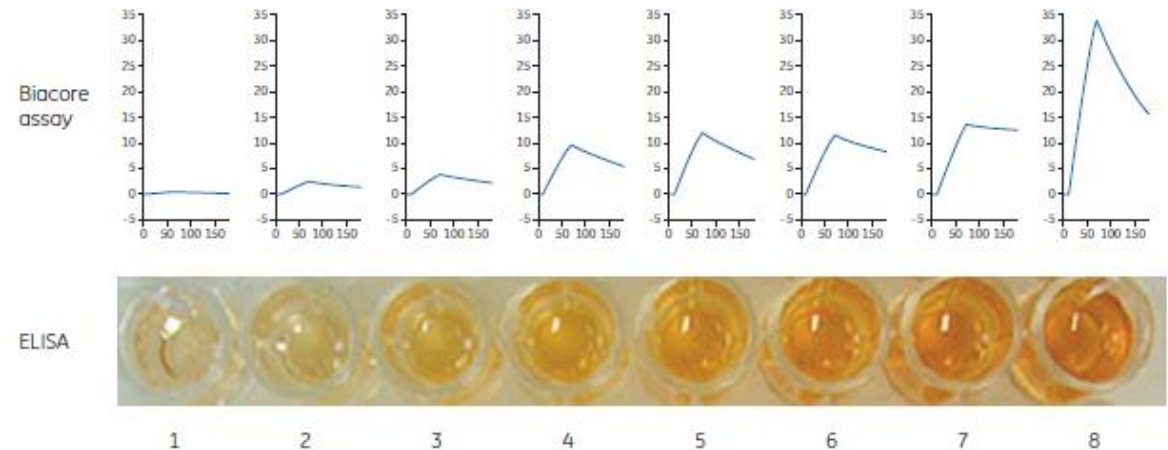
発現レベル

どのクローンが十分な量の抗体を産生するか？

Biacoreで抗体スクリーニングするメリットは？

インフォメーションリッチ・ロバスト

- リアルタイム、ノンラベルで測定・解析を実施できるため、期待する特性を持つ抗体を正確に評価しやすい。
- サンプルをセットすれば自動的に測定を実施できるため、作業者による結果のバラつきが起きにくい。
- Screening assay で簡易的な kinetics 情報を得た後は Characterization assay を実施して正確な kinetics 情報を得ることが多いため、検討初期から後期でも用いられる Biacore を使うことで、結果を同じ評価基準で見比べることができます。（共通言語化）



スループット (後述の測定系を参考にしていきます)

Report point-based screening	Biacore 8K+	Biacore 8K	Biacore T200
一度にマウントできる限界サンプル数 (96well plate (250uL) 使用時)	752 samples	240 samples	87 samples
上記サンプルの測定時間	約14時間	約5時間	約7時間
現実的な測定計画		2 Runs/day	2 Runs/day

2400 samples/week
※ 8K+ 最大3760

870 samples/week

Binding screen > Antibody screen
にテンプレートがあります

テンプレートプログラムを作成しました

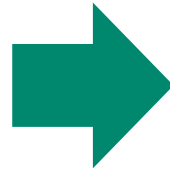
2

Assay format と解析

取り扱えるサンプルについて

流路が詰まるからクールドなサンプルは無理？

- ハイブリドーマの培養上清
- 大腸菌培養上清
- 血清



- 溶液が透明であればそのまま希釈して使用して構いません。通常、ランニング緩衝液（HBS-EP+推奨）にて2~20倍程度希釈して利用します。
- 一方で、論文では遠心（14000rpm, 5min程度）あるいはフィルトレーション操作により大きな粒子を除去しているものも見かけられます。
- 非特異的結合が起きる場合は NSB Reducer (BR100691) を終濃度 1mg/mLになるよう添加すれば抑制できる可能性があります。

フルサイズの抗体以外でも可能？

- Fab
- scFv
- その他何かしらのタグがあれば



抗体をリガンドとし、抗原をアナライトとする系を推奨します

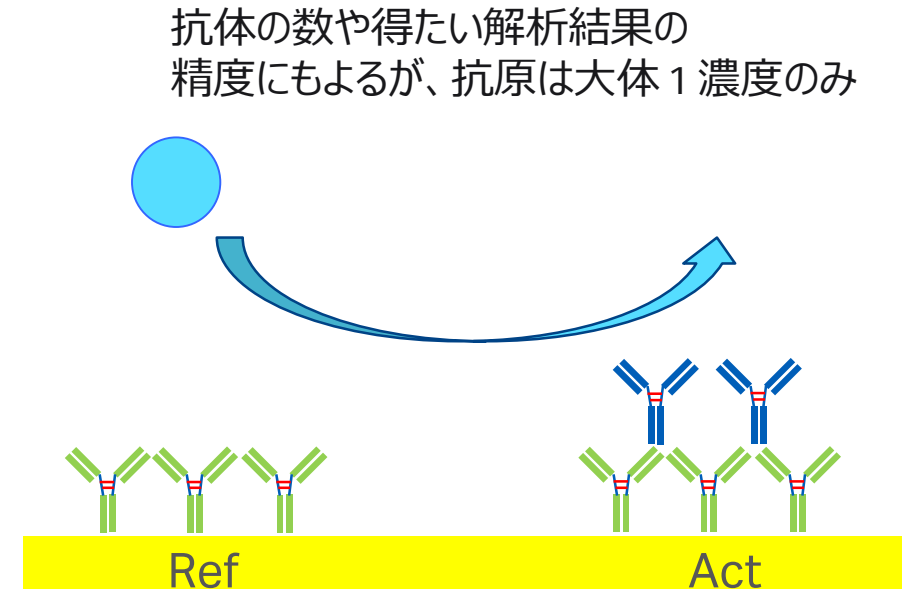
特徴

- 抗抗体やSensor Chip Protein Gなどでキャプチャーします。
- 抗体の濃度は不明であることが多いため、アナライトとしてしまうとkinetics解析ができません。
- 2価の抗体がアナライトの場合、off-rateによるランキング評価が難しくなります。

※抗原をリガンドとする系を否定するわけではありません。

サンプル量などとの兼ね合いでご自身にて決定してください。

ただしその場合は抗原のリガンド密度を低くすることを推奨します。



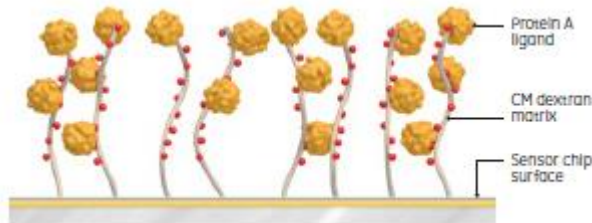
抗体（サンプル）はどうキャプチャーするべきか？

Sensor chip Protein A, G, (L)

大体これでOK

- Protein AやGやLが pre-immobilize されたチップです。
- 遠心済み培養上清などをそのまま添加することで抗体はキャプチャーされます。

Sensor chip	抗体種
Protein A	Human IgG1, IgG2 and IgG4 mammalian IgG Stability of mouse IgG1 is quite low, so kinetic studies challenging
Protein G	Human, rat, mouse, rabbit, sheep, goat, pig, cow, horse IgG Not bind chicken IgG, human IgA, IgD, IgE and IgM
Protein L (for yes/no screening)	Kappa light chain subtypes 1,3 and 4 Not bind bovine immunoglobulins (so useful for antibodies from serum based culture)

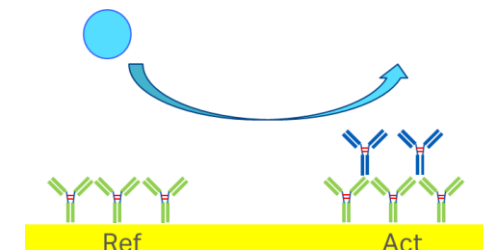


Cytiva

CM5 + 各種キャプチャーキット + Amine coupling kit

準備がやや大変...

- 弊社からは Mouse antibody capture kit, Human antibody capture kit, Human Fab capture kit を用意しています。その他の動物種については弊社以外の製品にて実施例があるものもございます（例：抗Rabbit抗体など）。
- Amine coupling Kit が必要、固定化の作業が必要などやや煩雑です。固定化量は5000RU以下程度にしてください。
- 通常 Reference cell にも抗抗体を固定化しますが、タンパク質フリーにしたいのであればあえて固定化しないことで対応可能です。
- Protein Gは Fatal Calf Serum と結合するためこちらが選ばれることもあります。



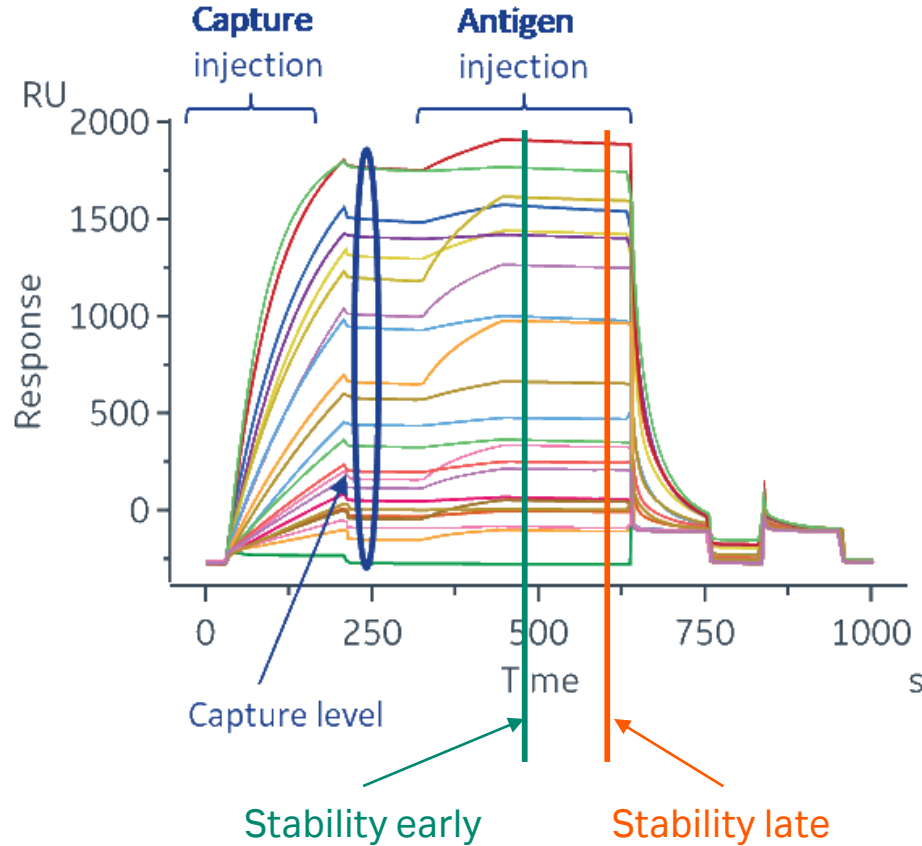
代表的な解析フォーマット

Blankサイクルを必要としない、Report point-based screening

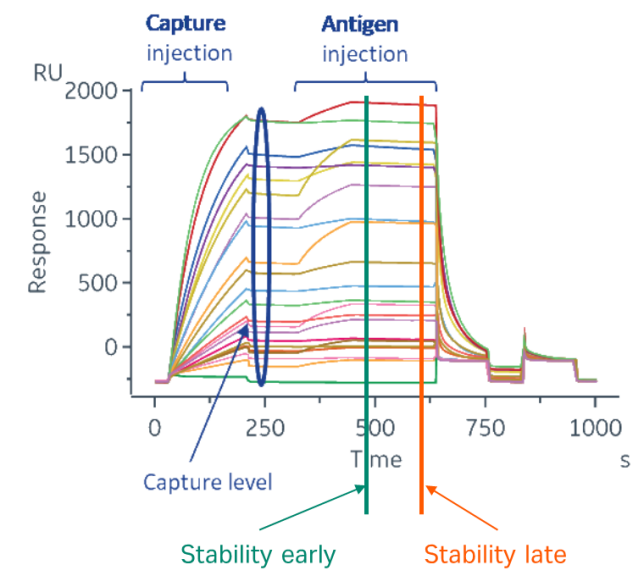
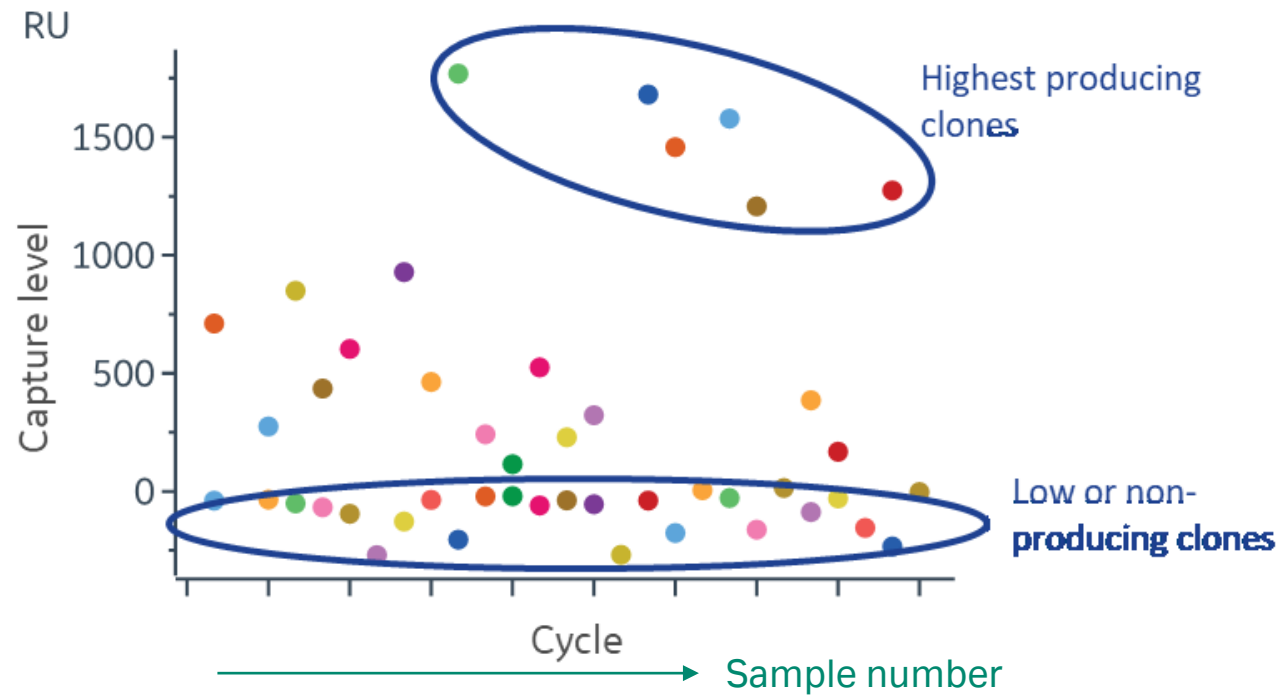
評価対象	評価内容
Capture level のプロット	そのクローンの抗体発現量
Stability early vs Capture level のプロット	抗体発現量は多くとも抗原との親和力が弱いクローンを見分ける
Stability early vs Stability late のプロット	複合体の安定性

Blankサイクルが必要な、Sensorgram-based screening

評価対象	評価内容
Off-rate ranking	<ul style="list-style-type: none"> Insight に標準搭載の 1:1 dissociation model 使用 抗原の濃度不要 Kinetics screeningよりも簡易
Kinetic screening	<ul style="list-style-type: none"> 1濃度の抗原だけでkaとkdを算出 On-Off rate map の評価可能

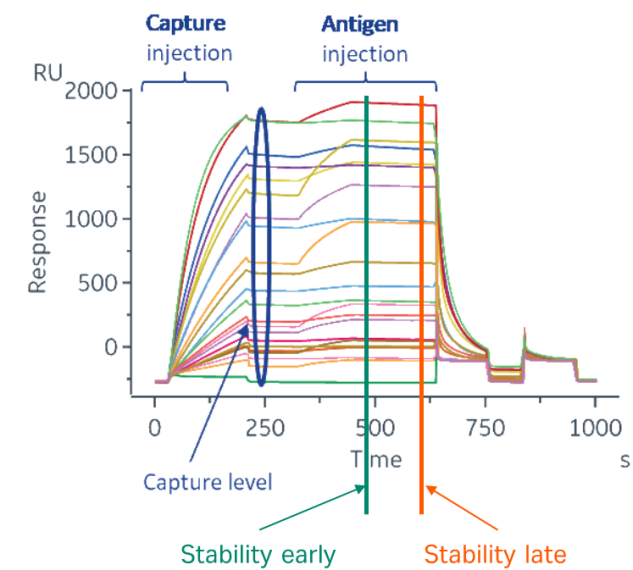
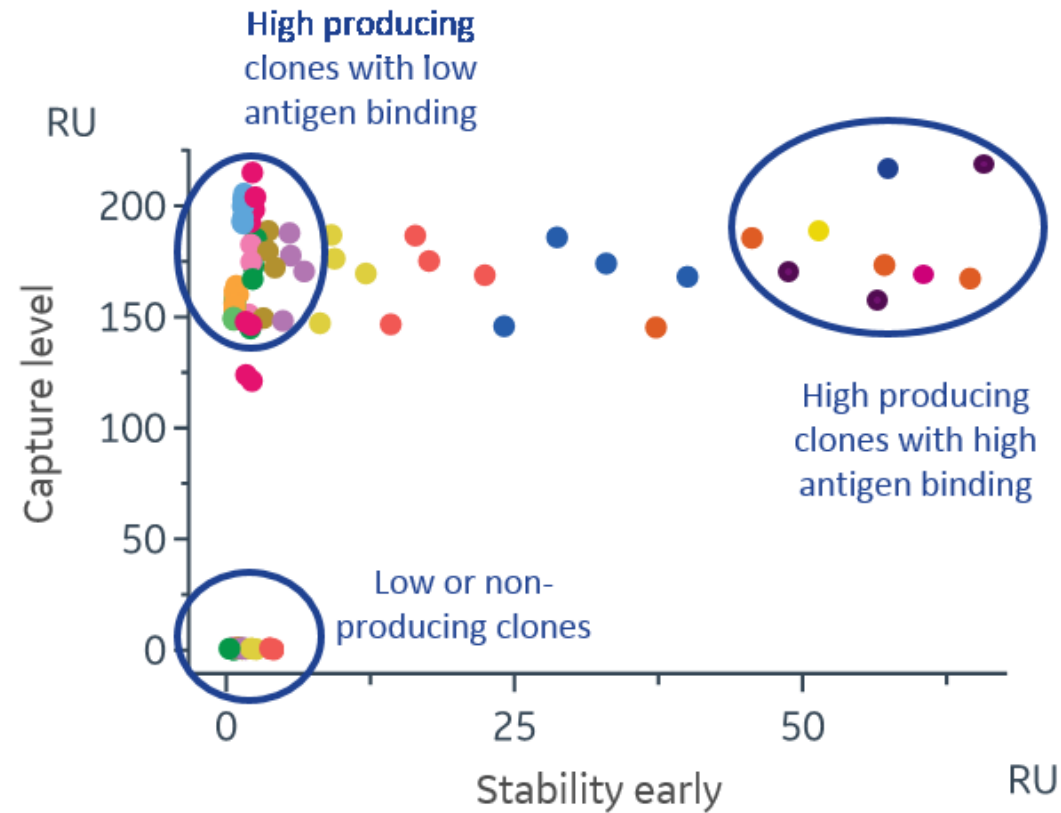


Capture levelのプロット



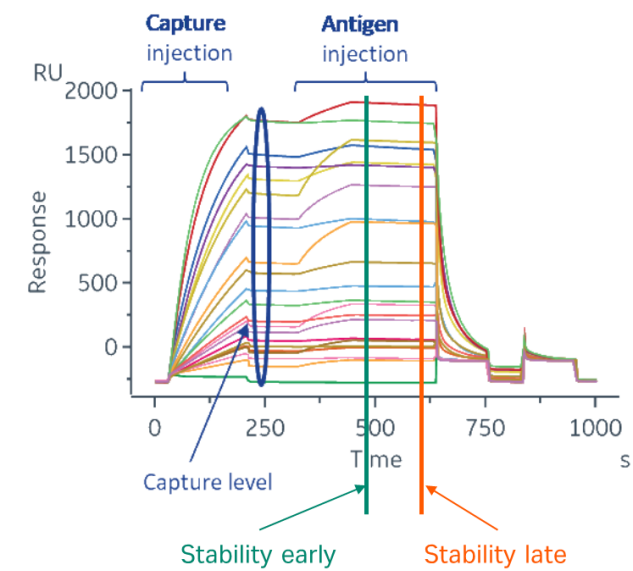
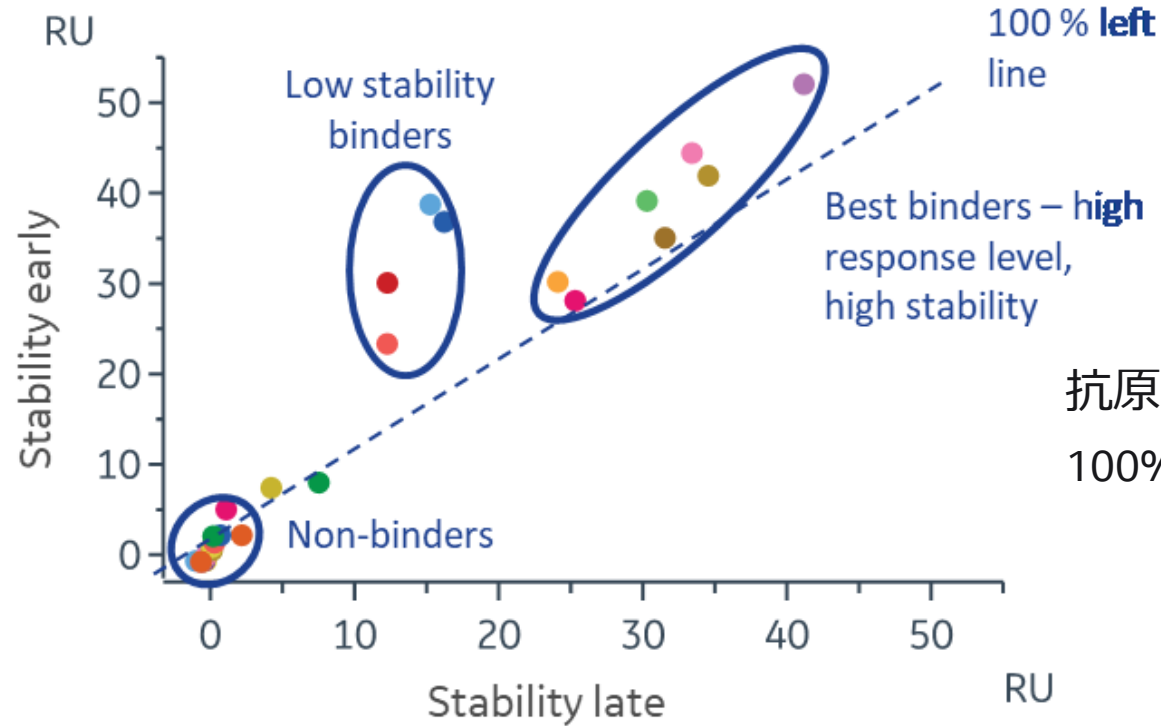
- 抗体発現量の多いクローンを選別することができます。

Stability early vs Capture level のプロット



- 抗原との結合量が少ないクローンを除外できます。

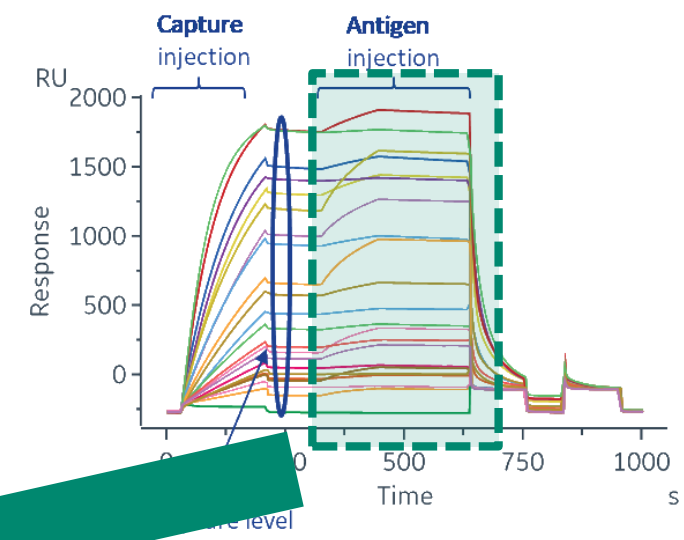
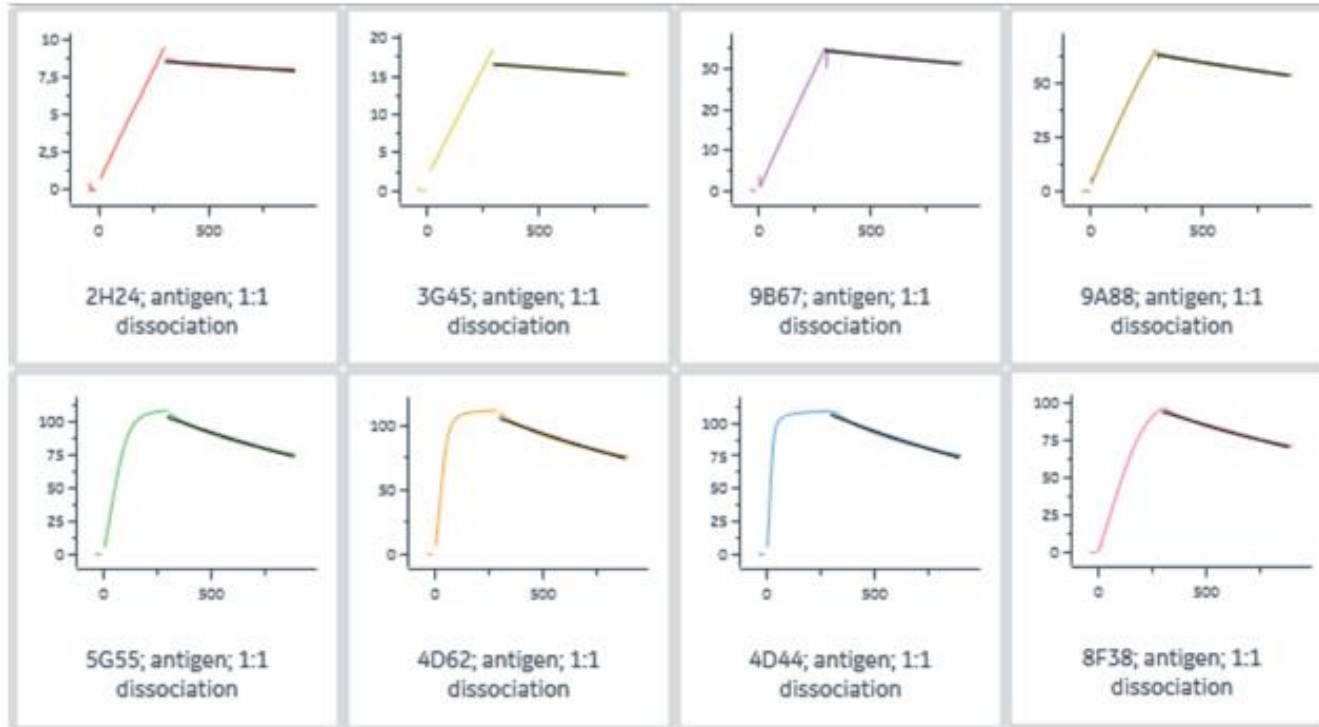
Stability early vs Stability late のプロット



抗原と結合後、その抗原が全く解離しないなら
100% left lineに乗ります。

- 複合体の安定性の良い抗体を選別できます。

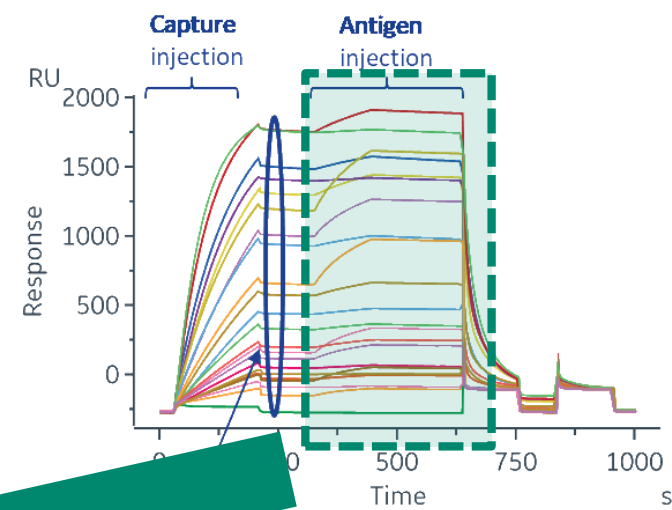
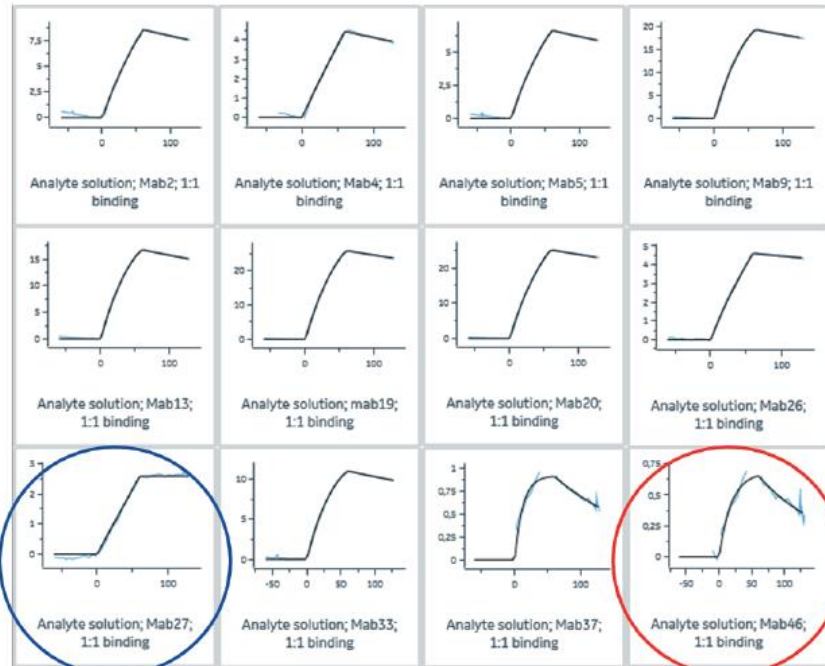
Off-rate ranking



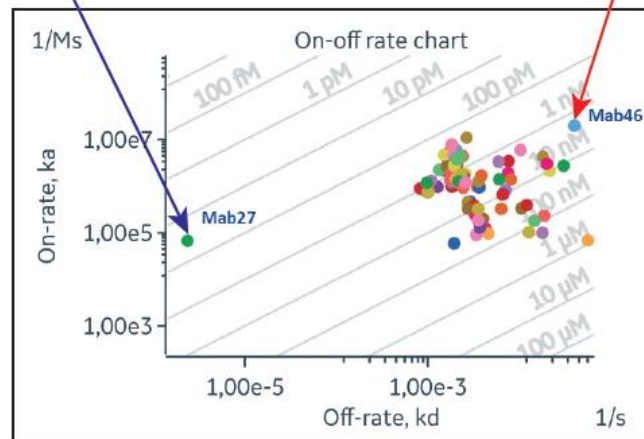
- **Blank (0濃度) のデータを差し引く必要があります。**
- 1:1 dissociation model で解析します。

• 複合体の安定性の良い抗体を選別できます。

Kinetic screening

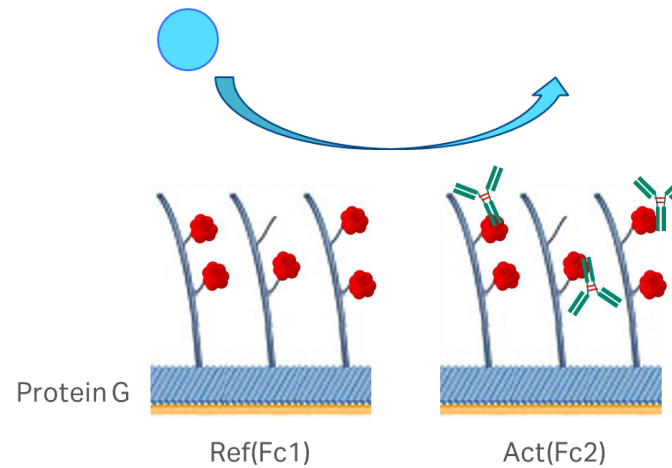


- **Blank (0濃度) のデータを差し引く必要があります。**
- 1:1 binding model で解析します。



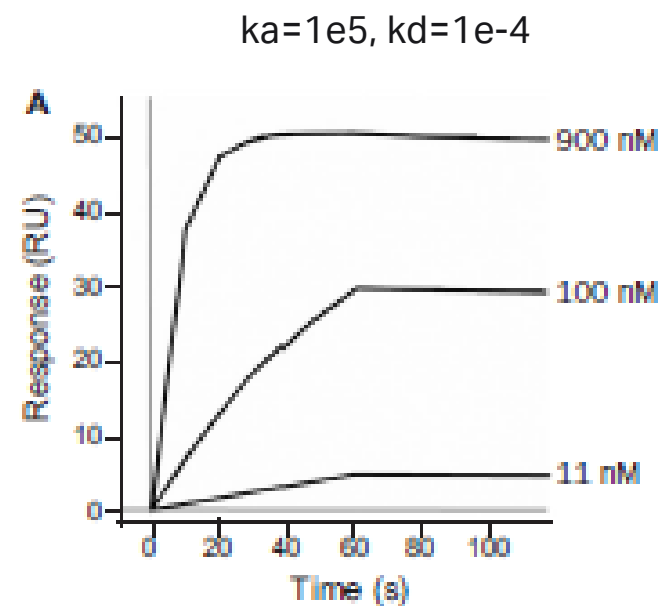
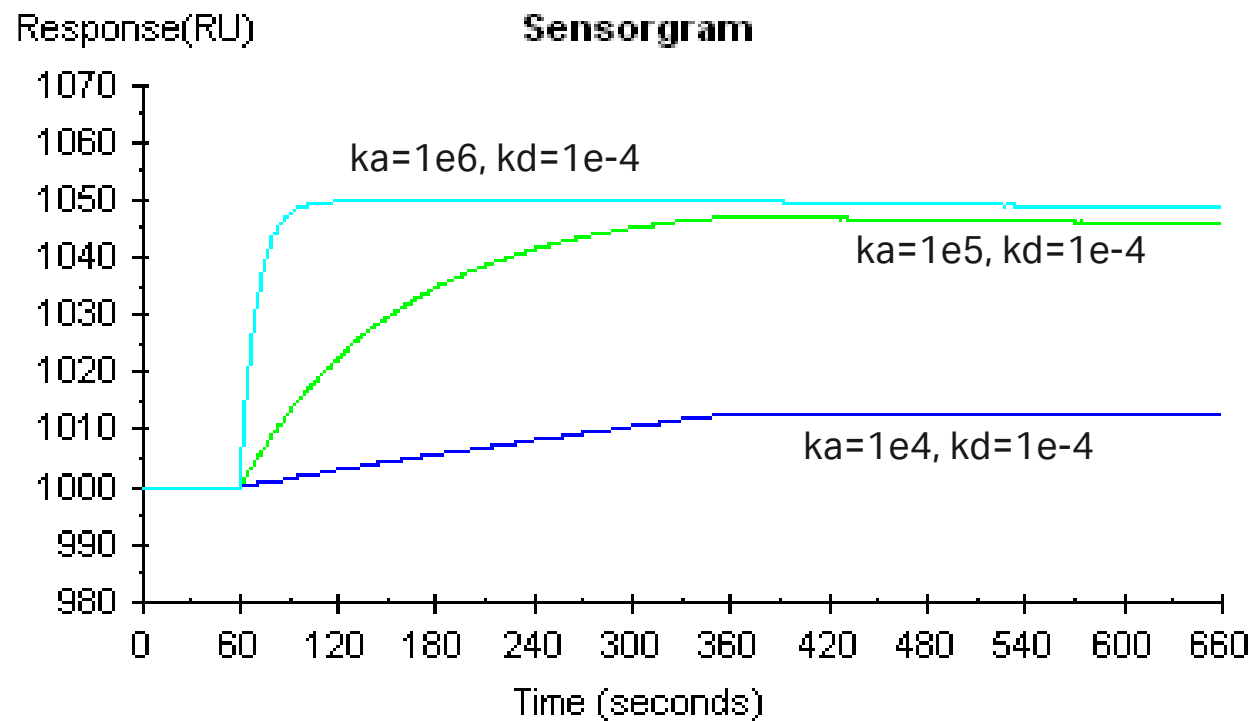
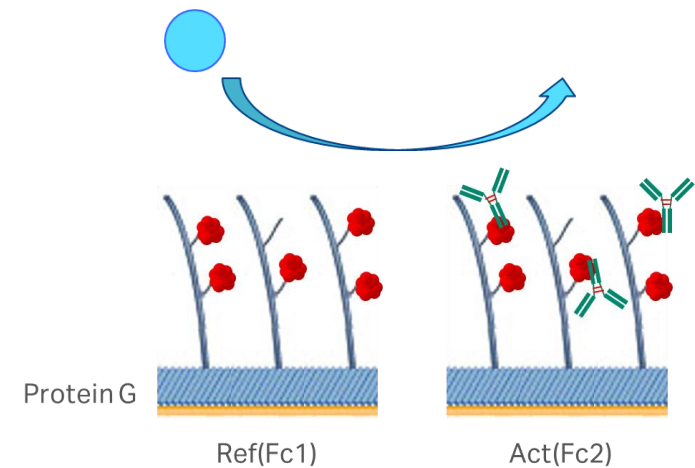
- On-Off rate map を描き、より詳細に望む特性の抗体を選別できます。

Assay setup



	Report point-based screening	Sensorgram-based screening
特徴	Blank が不要 <ul style="list-style-type: none"> • Capture level • Stability early, Stability late 	要Blank <ul style="list-style-type: none"> • Off-rate ranking • Kinetics screening
(Analyte) Flow rate	> 30 uL/min	> 30 uL/min
(Analyte) Injection type	Low sample consumption	High performance
(Analyte) Contact time	60 sec	60-120 sec
(Analyte) Dissociation time	120 sec	180 sec
(Analyte) Pooling	Optional	Optional
(Analyte) Concentration	Use the same conc. In all cycles	Use the same conc. In all cycles
(Ligand) Flow rate	10 uL/min	10 uL/min
(Ligand) Contact time	60-180 sec	60-180 sec

抗原の濃度は？



大体 100 nM 程度でOK

(Sensorgram-based screeningにおける) Blank の設定について

Blankを取る意味について

- Sensorgramを解析する際は、アナライタが0濃度のデータ (=Blank) を取得した上でアナライタ分子入りのデータからBlankを差し引くことで、ドリフト、機械的ノイズや非特異的結合の影響を抑える必要があります。
- その意味で、全ての抗体について①0濃度のデータと②アナライタ分子入りのデータを取得する必要があります。
- 一方で全抗体についてBlankを取るとスループットが悪くなりますので、「5-10サイクルおきに代表的な抗体をキャプチャーした上で0濃度を取り、それをBlankとする」という方法を取ることも可能です。

理想的な Setup

	Ligand	Analyte
Cycle 04	●	○ Blank
Cycle 05	●	●
Cycle 06	●	○
Cycle 07	●	●
Cycle 08	●	○
Cycle 09	●	●
Cycle 10	●	○
Cycle 11	●	●
Cycle 12	●	○
Cycle 13	●	●
Cycle 14	●	○
Cycle 15	●	●

スループット重視

	Ligand	Analyte
Cycle 04	● Rep	○ Blank
Cycle 05	●	●
Cycle 06	●	●
Cycle 07	●	●
Cycle 08	●	●
Cycle 09	●	●
Cycle 10	● Rep	○ Blank
Cycle 11	●	●
Cycle 12	●	●
Cycle 13	●	●
Cycle 14	●	●
Cycle 15	●	●

3

具体的なアプリケーション紹介

App Note 84

Early kinetic screening of hybridomas for confident antibody selection using Biacore A100

概要

- 抗ヒト抗体を固定化したチップに対し、ハイブリドーマ細胞培養上清を添加→抗原1濃度→再生を行っています。
- 抗体のキャプチャー量でノーマライズした後の Stability early と Stability late のプロットを描き、高発現株かつ複合体の安定性の高い株を選定。
- スクリーニング時の簡易的な k_a 、 k_d のデータと抗体精製後の詳細解析で改めて k_a 、 k_d を算出したときのデータにおいて、高親和性であればあまり差がないことを確認。

Early kinetic screening of hybridomas for confident antibody selection using Biacore A100

- Rapid, kinetic screening of mAbs direct from hybridoma samples
 - efficient screening rate of ~800 hybridomas/day
 - effective selection of mAbs with desired kinetic profiles
- High resolution kinetic characterization of selected mAbs
 - affinity and kinetic parameters determined directly from crude samples
 - dedicated evaluation software processes kinetic data in minutes
 - selection of candidates based on therapeutically relevant binding properties
 - parallel analysis under different buffer conditions for characterization of stability of antibody performance
- More confident mAb selection earlier in the development process compared to end-point assays such as ELISA

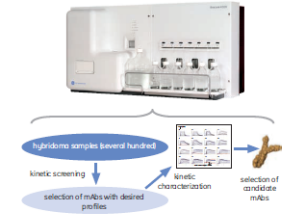


Figure 1. Biacore A100 offers rapid kinetic screening and high resolution kinetic characterization of mAbs direct from crude hybridoma samples in

Evaluation of screening results using report point values

Label-free interaction analysis produces a real-time plot of binding response against time (the *sensorgram*) over the entire course of binding and dissociation. Binding responses at specific selected times (report points) can be taken, to rapidly plot binding characteristics of interest (see *Monitoring Interactions* at the end of this note). Figure 3 shows a plot of responses from the late association phase (*binding_late* report point) from each mAb-antigen interaction recorded in the screening assays. Since the *binding_late* response is related to the association rate of the interaction and *stability_late* is inversely related to the dissociation rate, these two values provide a good overview of the kinetic profile of each mAb.

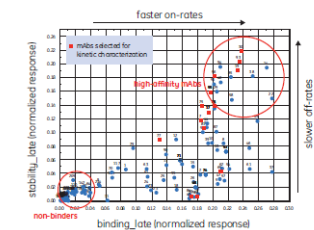


Figure 3. Rapid kinetic screening of hybridomas, evaluated by report point plot (responses normalized to capture level). Areas corresponding to low/non-binders and highest affinity, as well as the 16 mAbs selected for further characterization are indicated on the figure.

As seen in Figure 3, mAbs from the 384 hybridoma samples displayed a wide range of kinetic profiles. A number of negative hybridomas were supplied blind by Abgenix, and all of these were determined to be non-binders by the kinetic screening assay (i.e. no known false-positives were detected). Using this plot, 16 hybridomas were selected for comprehensive kinetic characterization, covering a wide range of association and dissociation properties among the positive samples.

Estimation of affinity & kinetic parameters from rapid kinetic screen

Single binding curves can be used to derive approximate k_a and k_d values and hence, estimate affinities, although fully quantitative, high-resolution kinetic characterization requires analysis of multiple sensorgrams derived from a concentration series of analyte. To obtain an estimate of the affinity/kinetic parameter ranges corresponding to the profiles observed in the report point plots, sensorgrams were therefore fitted to a 1:1 interaction model and the approximate rate constants used to generate an off/on-rate map (Figure 4). From this log-scale plot of estimated k_d against estimated k_a , a generally good correlation in kinetic profiles was observed compared to those indicated by the report point plot (Figure 3). The off/on-rate map also showed that many mAbs lay within an approximate affinity range of 1-10 nM and that the three highest affinity examples lay in the sub-nanomolar range.

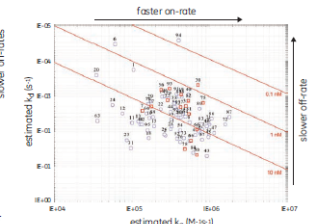


Figure 4. Rapid kinetic screening of hybridomas, evaluated by estimated rate constants. Log-scale plot of estimated k_d against estimated k_a . The isomeric lines corresponding to 10, 1, 0.1, 0.01 nM affinities are indicated by the red diagonals. Negative hybridomas and lower-level binders were excluded from this plot.

ent an important class whole biomedical research tools in the (as precision diagnostic reapeutics against a wide rances now enable the bodies directed against a) increasing demand for high quality binding data action.

lent, end-point Biacore™ systems for analysis of antibody based on functionally

App Note 79

Development of immunotherapeutics and immunization regimes

概要

- マウスにヒト IgE を免疫し、得られた抗IgE抗体を様々な方法で評価しています。
- IgEを固定化したチップに対しマウス血清を添加することで血清力価の経時的な検証、追加免疫の効果検証、マウスごとの個体差、親和性成熟の検証。さらに抗サブクラス抗体を添加することでサブクラスの確認。
- 免疫初期の親和性成熟が行われていない弱い抗体について、ELISAとBiacoreの検証結果の差について考察。

Application Note 79 Biacore systems

Development of immunotherapeutics and immunization regimes

Characterization of serum antibody responses during development of an anti-IgE based treatment for allergy and asthma, using Biacore T100

- Rapid, high information content characterization of immune responses direct from rat and primate serum
 - serum response patterns monitored over time following multiple immunizations
 - IgG subclass rapidly determined
 - qualitative changes derived from kinetic profiles
- Serum response assays correlated well with observed biological effects
- Significant advantages shown over ELISA
 - higher quality data: much lower inter- and intra-assay variations

Biacore T100

Summary

- The recombinant IgE-derived immunotherapeutic proteins developed by Resistoria Pharmaceuticals AB show excellent immunogenic properties in the two animal systems tested, providing the basis for an effective immunotherapeutic approach to the treatment of allergy and asthma.
- Biacore T100 provided high information content characterization of anti-IgE responses over time and in relation to immunization dosing and adjuvance regimes
 - Increased anti-IgE responses correlated strongly with reductions in circulating IgE levels, demonstrating neutralizing activity of the immunotherapeutic protein-induced antibodies
 - Subclass analysis showed a clear correlation between subclass switching patterns and effects on circulating IgE levels among different individuals
 - Rapid kinetic profiling of primate anti-IgE responses provided information on the quality of the antibody response over time and in relation to the dosing regime.
- Response data showed a good general agreement with ELISA, but showed a much higher level of reproducibility.

Methods

IgE and anti-IgE ELISAs were carried out using standard methodologies at Resistoria Pharmaceuticals AB. Biacore T100 assays were performed using Series 5 Sensor Chip CM5 and an assay temperature of 37°C. Standard amine coupling procedures were used to immobilize a commercial rat IgE protein (rat studj) or a Cynomolgus monkey IgE-derived recombinant protein (primate studj) onto the sensor surface. An overview of the assay setups for the two animal studies is shown in Figure 8.

Figure 8. Overview of assay setups used in the immune response assays. A) Rat study: commercial rat IgE protein immobilized to detect anti-IgE Abs in immunized animals. Further serial injections of IgG subclass-specific Abs is identified subclass of antigen-bound serum anti-IgE Abs. B) Primate study: recombinant Cynomolgus monkey IgE immobilized on the sensor surface. C) Deviation of data from sensograms. Binding of proteins on the sensor surface generates an SPR signal that is monitored in real time over the whole binding interaction (total interaction time: 30 minutes in the example shown). Anti-IgE and subclass responses were determined from single report points, taken from specific time-points on the sensograms. Qualitative assessment of Abs was based on slopes taken from the early dissociation phase. In practice, the response level and dissociation profiling data were obtained from separate experiments, with sensor surface preparation and other conditions tailored for the specific assay.

Biacore

App Note 28-9777-72AA

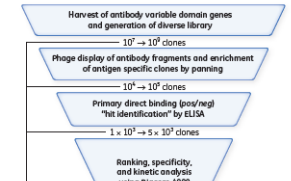
Resolving a bottleneck in screening and characterization of recombinant antibody fragments using Biacore 4000

概要

- 抗HAタグ抗体を固定化したチップに各scFvをキャプチャー、さらにトロポニンを追加した後に再生しています。
- Stability early と Stability late のプロット、Capture level での評価も組み合わせています。
- Characterization では25°Cと37°Cで取得したka、kdのプロットを描き、温度安定性の良い抗体を選別している点がユニークです。

Resolving a bottleneck in screening and characterization of recombinant antibody fragments using Biacore 4000

Antibodies and their derivatives are increasingly important as biotherapeutics, precision diagnostics, and essential tools for biological research. Techniques to screen and characterize large numbers of antibodies and provide confident selection of the best candidates for development are therefore essential. Biacore 4000 equipped with Biacore 4000 Antibody Extension Package has been used for efficient screening and characterization of antibody fragments for diagnostic purposes. Ten 96-well plates containing avian scFv fragments in crude cell lysates were screened and ranked in less than 20 h. A subset from this screen was selected for analysis with the 2-over-2 kinetics approach. This method



Results and discussion

Screening of scFv antibody fragments based on binding stability

A panel of 960 scFv fragments specific to human cardiac troponin I was screened using the assay setup described in Figure 2. Sensorgrams from 120 samples with the report points indicated as colored bars are shown in Figure 4.

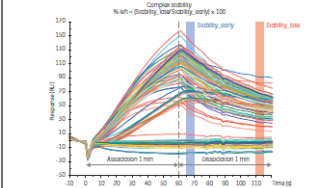


Fig 4. Sensorgrams from 120 samples showing report points used for ranking.

Figure 5 shows a scatterplot of the report points stability_early plotted against stability_late. This type of plot can be used to rank binders based on binding stability. The figure shows two main groups of scFvs: non-binders close to the origin and binders higher along the diagonal. The best binders, with a high binding stability and slow dissociation, are shown as blue squares. In total, 960 samples were analyzed and ranked with respect to binding stability in 18 h. The data evaluation was accomplished in less than one hour.

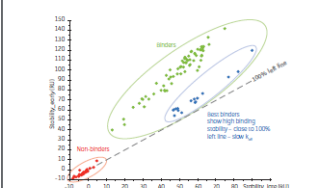


Fig 5. Stability_early vs Stability_late plot for identification of stable binders (best binders shown as blue squares, green binders are not as stable).

Importantly, Biacore analysis of antibodies and their fragments allows both the expression levels of the clones and quality of the antibodies to be measured simultaneously. Figure 6 shows an example of how report point data from the scFv screen are plotted in Spotfire, providing an excellent overview of the performance of different clones.

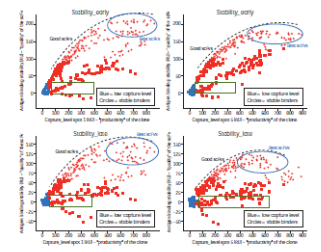


Fig 6. Overview of the scFv screening. Antigen binding stability, stability_early (top) and stability_late (bottom), is plotted against capture level in spot 1 (left) and spot 3 (right). Wells without scFv (blue), wells with significant capture (red), and scFvs showing a stable antigen response (circles) are displayed. The green box indicates scFvs that are captured, but do not bind to the antigen. The best scFv clones show high capture level and high binding stability (blue circles).

The amount of captured scFv on spot 1 and 5 in the four flow cells is plotted on the x-axis, and indicates the capture level or expression level of the different clones. After scFv has been captured, antigen is injected over the spots. Response levels early and late in antigen dissociation are plotted on the y-axis to show the quality of the interaction of captured scFv with antigen. Uninteresting clones are distinguished from the good clones (close to the dashed lines), and the best clones show both high capture level and binding stability (slow off-rates). These are important criteria when selecting high quality antibodies and antibody fragments for further development, and were applied to the scFvs selected for 2-over-2 kinetic characterization.

Characterization with 2-over-2 kinetics

Obtaining kinetics data early in the development process enables better-informed selection since kinetic properties have consequences for both therapeutic and diagnostic agents. The 2-over-2 kinetics approach is particularly suitable for providing kinetic information already during screening, as the data can be obtained in half the time using significantly less reagents and sample volumes.

Evaluation was performed with the kinetic evolution tool in Biacore 4000 Evaluation Software, by combined kinetic fitting of the sensorgram data from spots 1-2 and 4-5, respectively. The evolution combines the different surface densities with the different analyte concentrations in one fit.

To investigate temperature stability of the binding, all samples have been analyzed at 25°C (Fig 7A) and 37°C (Fig 7B). Kinetic constants from the two temperatures were exported to Spotfire for further analysis. Temperature-stable, high-affinity scFvs were identified in Spotfire using on/off-rate maps (Fig 8).

1 x 10⁵ clones
 1 x 10² clones
 studied at each screening stage
 less for selecting recombinant antibodies
 for secondary screening, has the
 analysis bottleneck and provides high
 ic data.
 r (prostate-specific antigen, PSA)
 ostatic reagents, ideal antibodies
 and a high affinity that is

Assessing long-term glucose regulation by measurement of glycated hemoglobin using Biacore 8K

概要

- Roche Diagnostics社のBiacore8K検証データ。
- Screening 後の Characterization について言及。
- 抗Rabbit抗体を固定化したチップに対して精製済みの抗体をキャプチャー、続いて5濃度分のペプチドを添加しkinetics解析を実施。
- 詳細解析にもかかわらずスループットも高い。

Assessing long-term glucose regulation by measurement of glycated hemoglobin using Biacore™ 8K

Persistently elevated levels of blood glucose lead to irreversible attachment of glucose molecules to hemoglobin in red blood cells. The longer the hyperglycemia occurs, the more glucose binds to hemoglobin. By measuring the binding of antibodies that recognize glycated hemoglobin

Biacore systems has become an indispensable process standard in the early development of mAbs for diagnostic and pharmaceutical applications. In particular, kinetic antibody screening is established in the diagnostic antibody production process. Kinetic screening resolves

A Biacore Series S Sensor Chip CM5 was mounted into the Biacore 8K system and was normalized in instrument buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% w/v Tween™ 20) according to the manufacturer's instructions. Samples were prepared in system buffer supplemented with 1 mg/mL of carboxymethyl-dextran (CMD, Sigma-Aldrich, code no. 86524). Samples were analyzed at 37°C. Approximately 12 000 RU goat anti-rabbit Fc fragment-specific antibody (GARbFcγ, Jackson ImmunoResearch Laboratories Inc, code no. 111-005-046) was immobilized according to the manufacturer's instructions using EDC/NHS coupling in both flow cells and in all eight channels. The sensor surface was finally saturated with 1 M ethanolamine. The binding kinetics of the respective rabbit mAbs against an HbA1c peptide analyte A01, relative molecular mass [M_r] of 1200) were generated and evaluated. The experimental assay setup is shown in Figure 1. Antibodies were injected at 200 nM concentration for 1 min at 10 μL/min. After capture, the flow rate was increased to 80 μL/min. Analyte A01 was injected for 3 min in a concentration series of 0 (buffer control), 3, 10, 30 (in duplicate), as well as 90 and 270 nM. The analyte dissociation was monitored for 5 min. After each analyte injection, the antibody capture system was fully regenerated by a 15 s injection of HBS-ET buffer at 20 μL/min; a 20 s injection at 20 μL/min with 10 mM glycine buffer pH 2.0, and two injections for 1 min at 20 μL/min with 10 mM glycine pH 2.25. Kinetic signatures were evaluated using a 1:1 binding model with local R_{max}.

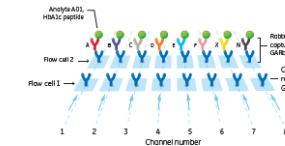


Fig 1. Experimental assay setup for kinetic evaluation of six rabbit mAbs (A01 to A06) with specificity for human HbA1c peptide analyte A01. GARbFcγ was immobilized in flow cells 1 and 2 and in all eight channels. The rabbit mAbs were captured on GARbFcγ in flow cell 2. Human HbA1c was injected in both cells. Rabbit mAb X with unrelated target binding function and rabbit normal IgG1G2 were used as controls.

Binding specificity of rabbit mAb to various human HbA1c peptide derivatives

After the kinetic experiments, mAb A was selected to determine specificity against seven human HbA1c peptide analytes with M_r between 1100 and 1500 and a buffer control (Fig 2). The peptide analytes differed in their amino acid composition, sequence length, glycosylation, or N-/C-terminal chemical modifications. For example, A01 is glycosylated HbA1c positive control peptide and A02 and A05 are nonglycosylated HbA1c peptide negative controls. A07 is the HbA1A2 cross-reactive negative control. A03, A04, and A06 are positive controls for HbA1c peptide derivatives with chemical modifications or prolonged peptide sequences to avoid N- or C-terminal antibody peptide binding. Analytes A01, A03, A04, A05, and A06 were injected in a concentration series as previously described. Analytes A02 and A07 were injected at a higher concentration series from 0, 10, 30, 90 nM (in duplicate), and 270 nM to 810 nM to better identify unwanted cross-reactive binding.

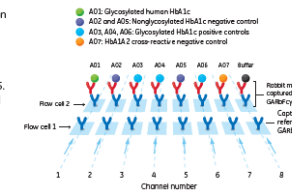


Fig 2. Experimental assay setup for the specificity test of mAb A against seven human HbA1c peptide analytes, A01 to A07. GARbFcγ was immobilized in flow cells 1 and 2 and in all eight channels. Rabbit mAb A was captured on GARbFcγ in flow cell 2. Human HbA1c peptide analyte was injected in both cells.

Kinetic Screening in the Antibody Development Process

Antibody Methods and Protocols pp 171-18

Chapter 11

Kinetic Screening in the Antibody Development Process

Michael Schröml and Matthias Biehl

Abstract

Kinetic screening is of paramount importance when it is to select custom-made antibodies, tailored for their respective scientific, diagnostic, or pharmaceutical application. Here a kinetic screening protocol is presented. The assay is based on an ELISA method using a single injection. The method enables the screening of antibodies with different kinetic signatures.

if life ($t_{1/2, \text{off}}$), Binding Late,

4) means to select antigenic kinetic rate properties, dissociation constant K_d (M), in a workflow, where it ELISA-based screening, information about an antigen is of potentially suitable

asson resonance (SPR) uses six easy-to-access pictures to quickly select sample sets. The screening captured on the sensor binding signal at the end

Molecular Biology, vol. 901,

概要

- Roche Diagnostics社の抗体スクリーニング方法の紹介です。
- 基本的な戦略は同じながらも、抗体をキャプチャーする際にCMDを加えている点などで差異があります。
- 評価方法もユニークで、 $t_{1/2}$ での足切り、キャプチャーされた抗体あたりの抗原結合比などを利用していません。
- Methods Mol Biol. 2012;901:183-94
Temperature-dependent antibody kinetics as a tool in antibody lead selectionでは温度変化による k_a 、 k_d のパラメータの変化から抗体を3つに分類するなど新しい目線での評価がなされています。

11 Kinetic Screening in the Antibody Development Process 177

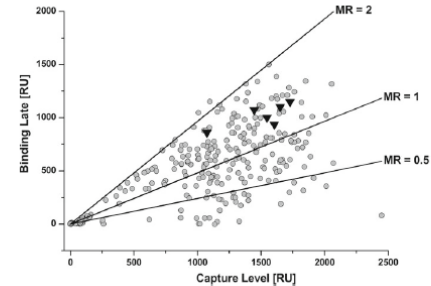


Fig. 3. Exemplary valence analysis of the data set from Fig. 2. Binding Late (RU) is plotted over the antibody Capture Level (RU). Corridors are formed by lines, indicating areas of the same Molar Ratios. The lines help to quickly classify antibodies according to their valences. The antibodies populate all corridors and therefore bind to their 72-kDa antigen with different valences. Black triangles indicate the selected clone cultures from Fig. 2.

To graphically visualize the valence analysis, Binding Late is plotted over the antibody Capture Level. Valence corridors, calculated using the MR formula, allocate the antibodies according to their virtual Binding Late values (Fig. 3), e.g., for the plotting of the MR=2 trendline use the formula: BL at MR(2) = (MW(antigen) \times 2 \times Capture Level (RU)) / MW (antibody)). Just replace the Molar Ratio values to calculate different trendlines.

When the antibody shows a MR=1, it binds to an antigen with single valence. Obviously, there is some steric hindrance, which avoids full bivalent antibody binding.

When the antibody shows MR=2, it is able to simultaneously bind to two antigens.

Here antibodies in the range of MR=1 and MR=2 were selected, when they showed a suitable BL/SL ratio (Fig. 3) at the same time. For further details about the valence analysis see Notes 9 and 11.

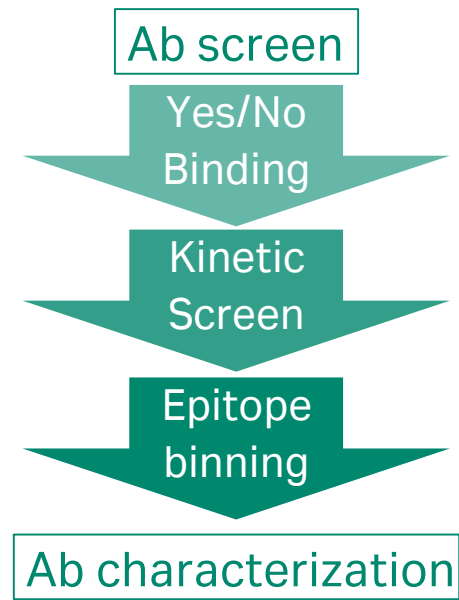
3.5. Data Analysis for k_d Ranking

If possible, calculate the "off rate", which means the antigen dissociation rate constant k_d (1/s) (6). The dissociation rate constant k_d describes the decay of complexes per second. k_d is calculated from a linear regression fitting of the antigen dissociation phase, which is a function of the Biaevaluation software. Using the dissociation rate constant, the complex half life $t_{1/2, \text{off}}$ in minutes

Epitope binning analysis for further differentiation of therapeutic antibodies and diagnostic reagents

概要

- BiacoreでEGFRに対する抗体のEpitope mapping (Sandwich, Premix) を行っています。
- 既報のX線結晶構造解析によると抗EGFR抗体のマツズマブとセツキシマブとは異なるエピトープのためサンドイッチ構造を取ることができるはずですが、Epitope binningの解析結果から、2つの抗体が溶液中で同時に存在しているときはお互いの結合を阻害していることが示唆されました。



Epitope binning analysis for further differentiation of therapeutic antibodies and diagnostic reagents
 Linnea Nygren-Baboi, Michael Murphy, and Anna Jansson
 GE Healthcare Bio-Sciences AB, SE-751 84 Uppsala, Sweden

Background

Our interest with this study was to establish a well characterized and easily accessible model system to be used for epitope binning assays using Biacore® and/or surface plasmon resonance (SPR) systems. Our criteria for the model system were that a monoclonal antigen and at least two antibodies should bind to the antigen simultaneously. The system we analyzed was epidermal growth factor receptor (EGFR) and three therapeutic antibodies targeting the extracellular domain of EGFR. The antibodies were panitumumab (Vectibix®), cetuximab (Erbitux®), and matuzumab (Cytuxen®). According to the previously published X-ray crystal structure, the Pan- fragment of matuzumab (Pan2000) interacts with an epitope adjacent to the cetuximab epitope and should thus be able to form a sandwich complex with cetuximab. Panitumumab and cetuximab, on the other hand, recognize the same epitope and should thus block each other. The major aim of the study was to evaluate the results from epitope binning using sandwich and premix assays and our interpretation of the findings.

Epitope binning EGFR (Sandwich)

Experimental conditions

- Antigen: epidermal growth factor (EGF), recombinant (EGF-EGF), and recombinant (EGF-EGF) in water (0.1M NaCl, 0.05M HEPES, pH 7.4)
- Antibody 1: Pan-EGFR
- Antibody 2: Cetuximab
- Antibody 3: Panitumumab
- Antibody concentrations: 4 nM, 40 nM, and 400 nM
- Flow rate: 30 µL/min
- Regeneration: 2 M NaOH, 10 min in buffer pH 2.0 (PharMA)

Results

The binding was observed in all of the combinations tested. The results from the different assays are shown in Figure 1. The results show that Pan-EGFR and Cetuximab can form a sandwich complex, while Panitumumab and Cetuximab cannot. This was not observed for Panitumumab.

Epidermal growth factor receptor (EGFR)

EGFR is a transmembrane protein and a member of the ErbB family of receptors, a subfamily of other closely related receptor tyrosine kinases: EGFR (ErbB-1, HER2/neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4). Mutations affecting EGFR expression or activity could result in different cancer types. Anti-EGFR monoclonal antibodies for therapeutic use block the extracellular domain of EGFR and block ligand-induced EGFR tyrosine kinase activation. In this study, we used the extracellular domain (EGF-EGF) of human EGFR (ErbB1 domain).

Epitope binning EGFR (Premix)

Experimental conditions

- Antigen: recombinant EGF (EGF-EGF), recombinant (EGF-EGF), recombinant (EGF-EGF), recombinant (EGF-EGF) in water (0.1M NaCl, 0.05M HEPES, pH 7.4)
- Antibody 1: Pan-EGFR
- Antibody 2: Cetuximab
- Antibody 3: Panitumumab
- Antibody concentrations: 4 nM, 40 nM, and 400 nM
- Flow rate: 30 µL/min
- Regeneration: 2 M NaOH, 10 min in buffer pH 2.0 (PharMA)

Results

The results show that all three of the antibodies were blocking each other.

Sandwich assay

In this assay, the first antibody is immobilized on the sensor chip. The second antibody is then injected and binds to the antigen. The third antibody is then injected and binds to the antigen. The results show that Pan-EGFR and Cetuximab can form a sandwich complex, while Panitumumab and Cetuximab cannot.

Premix assay

In this assay, the antigen is immobilized on the sensor chip. The antibodies are then injected and bind to the antigen. The results show that all three of the antibodies were blocking each other.

Fab-EGFR complex

X-ray crystallographic data of the Fab fragments of cetuximab (C190) and matuzumab (C200) in complex with the EGF extracellular domain (EGF-EGF) are shown. The epitopes are shown in red and blue. The results show that Pan-EGFR and Cetuximab can form a sandwich complex, while Panitumumab and Cetuximab cannot.

Results presented as heat map and bin chart

The heat map shows the results from the different assays. The bin chart shows the results from the different assays. The results show that Pan-EGFR and Cetuximab can form a sandwich complex, while Panitumumab and Cetuximab cannot.

References

- Schmidt, L. et al. Molecular binding of EGFR presents the conformational requirements for the extracellular domain of EGFR. *PLoS ONE* 10(12): e0183888 (2017).
- Li, L. et al. Structural basis for inhibition of epidermal growth factor receptor by panitumumab. *Chem Biol* 16(11): 1201-1211 (2013).
- Schmidt, L. et al. Epitope binning of antibodies: conformational requirements for the extracellular domain of EGFR. *PLoS ONE* 10(12): e0183888 (2017).

Conclusions

Combination of data from different X-ray structures may not represent the "real life" scenario. The results from the different assays show that Pan-EGFR and Cetuximab can form a sandwich complex, while Panitumumab and Cetuximab cannot. This was not observed for Panitumumab. The model system did not fulfill our criteria as all antibodies recognized the same epitope or shared a common epitope. Ability to measure binding at low immobilization levels and low sample concentrations requires a sensitive instrument — in this case, Biacore S—no distinguishable binding from unbound antibodies. The response may occur when working with high surface and sample concentrations.

4

まとめ

Biacoreで抗体をスクリーニングするにあたってのポイント

- + 抗体はクルードサンプルが良い（が、そのままではなく一応遠心分離した方が無難）
- + 抗体をリガンドにするため Protein G チップなどでキャプチャー法を取る
- + 抗原は1濃度（100nM程度）で十分、事前にプログラムで必要量を確認しておく
- + Blank は必ずしも必要なし（特にReport point-based screen）
- + Screening で選別が終わったら Characterization（精製して詳細解析）へ

■ 抗体スクリーニングの完了



Thank you

Masami Koinuma

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【基礎編】ウェスタンブロットティングの基礎からトラブルシューティングまで

Cytiva アプリケーションスペシャリスト 川上 裕貴

 [Register](#)  2020年8月26日  15:00～15:30

CONTENTS

ウェスタンブロットティングは一般的な実験手法ですが、思うような実験結果が出ないこともあります。

本ウェビナーでは、【基礎編】からプロでも役に立つ【実践編】まで、さまざまな失敗例をもとにトラブルシューティングについてお伝えします。

これからウェスタンブロットティングをはじめの方、既の実験を行っているがうまく検出できない方は、ぜひご参加ください。

【基礎編】




ウェスタンブロットティング原理

試薬・抗体濃度の選択

バックグラウンドの低減方法

細胞画像解析への新たなアプローチ～Deep Learning画像認識、Machine Learning細胞分類、3D Volume 解析

Cytiva アプリケーションスペシャリスト 高田 元

 [Register](#)  2020年8月27日  15:00～15:50

CONTENTS

細胞画像解析の課題として、1) S/Nの低い蛍光画像や明視野画像、また高密度な細胞を正確に認識させることが難しい、2) いろいろな表情を持つ正常細胞と疾患細胞などの細胞分類が困難、3) 3D培養系での多検体解析をどのように進めればいいのか、といった声を聴きます。そこで、今回はIN Carta image analysis softwareを用いることで可能となる新しい細胞画像解析アプローチをご案内します。

