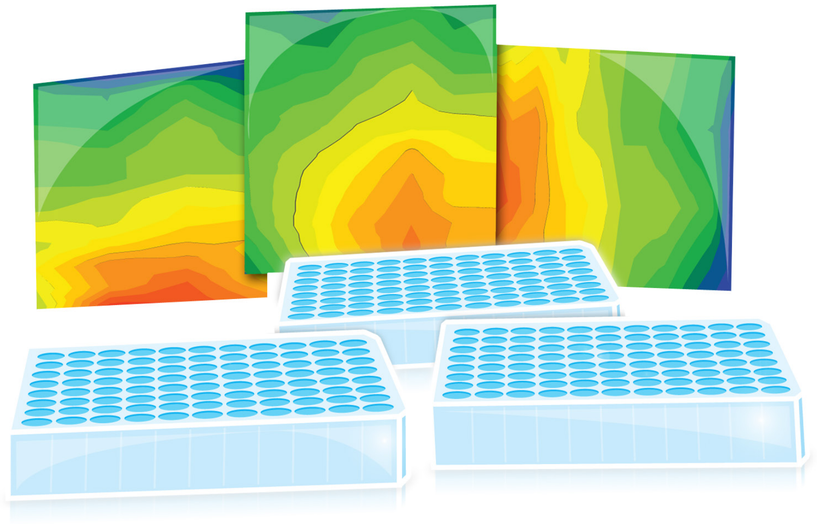
Principles and methods

High-throughput process development with PreDictor plates



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Contents

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Introduction

Time-to-clinic and time-to-market are two key factors for successful biopharmaceutical development. Efficient development of the manufacturing process is a crucial component of the overall project plan. However, screening optimal process conditions can be time-consuming and tedious. In addition, steadily increasing demands from regulatory authorities for better understanding and control of manufacturing processes put even more pressure on development work. Employing high-throughput tools for process development helps address these challenges.

High-throughput process development (HTPD) shortens development time at the same time as it increases the amount of information available during early process development. Evaluating chromatographic conditions can be performed in parallel using 96-well filter plates. As a result, a large number of experimental conditions can be evaluated simultaneously. This allows a large experimental space to be characterized and supports the definition of a well-established process design space where process parameters that need to be monitored and controlled are understood (Fig 1).

This handbook focuses on process development work in PreDictor™ 96-well filter plates, which are prefilled with chromatography media (resins). The handbook describes how results obtained using PreDictor plates can be used to define process conditions for large-scale chromatography. Applications of PreDictor plates are provided, as well as practical hints and tips. Assist software, dedicated to allow high-throughput methodology using PreDictor plates, is also described.

Symbols

This symbol indicates general advice on how to improve procedures or recommends measures to take in specific situations

01

Process development in PreDictor 96-well plates

1.1 Why use PreDictor 96-well plates as part of a process development workflow?

In the past, process development was either performed using tedious and time-consuming column experiments, or such experiments were simply not performed. This resulted in a less well understood and less well optimized process. With PreDictor plates, many types of experiments can be performed in parallel, which result in significant time-savings and lower consumption of sample and other reagents. The high-throughput format allows rapid screening of a wide range of conditions and thus allows generation of large amounts of valuable process data.

The workflow in a PreDictor experiment comprises the same steps as any column experiment. When using PreDictor plates, the fundamental interactions between the chromatography medium and the target molecule are the same as in chromatography columns. Basic concepts such as mass balance, rate of uptake, and adsorption isotherms are the same for PreDictor plates and chromatography columns. Successful application of PreDictor experiments does not require in-depth knowledge of these concepts nor of the equations presented later in this handbook. The applications presented show that data obtained in PreDictor experiments are relevant for full-scale process design and characterization.

1.2 Screening experiments in PreDictor plates vs small-scale columns

High-throughput tools such as PreDictor plates and Assist software are suitable for early chromatographic screening experiments. They can be used for the initial screening of process conditions, or for a more thorough investigation of a defined space as a basis for detailed process understanding and/or robustness studies.

After scouting and screening with PreDictor plates, verification and fine-tuning are still performed with ÄKTA™ design systems such as ÄKTA avant™ 25 at a smaller scale than the expected manufacturing process, see Section 9. Prepacked formats such as HiScreen™ 10 cm bed height columns are recommended, or HiTrap™ 1 mL and 5 mL columns if sample volumes are low, see *Ordering information*.

Figure 1 shows a conceptual workflow for process development with PreDictor plates as the starting point.

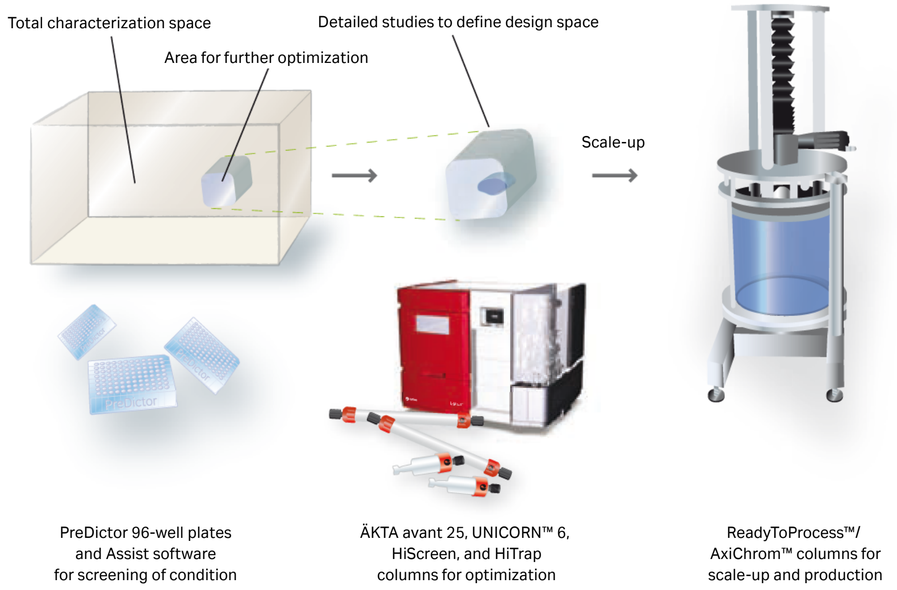


Fig 1. Conceptual workflow for process development.

1.3 What types of studies can be performed?

PreDictor plates are used to:

- Screen chromatographic conditions. This includes all parts of the chromatographic cycle: binding, wash, elution, and cleaning-in-place. Screening can be performed for different media simultaneously, or for a single selected medium
- Determine adsorption isotherms. Briefly, the adsorption isotherm describes the relationship between the concentrations of protein(s) in the liquid and solid phases at equilibrium under a given set of experimental conditions. This helps understand and correctly describe what happens during protein uptake under different conditions. It also helps to determine optimal phase ratios (ratio between sample volume and volume of chromatography medium) for capacity, wash, or elution studies.

Typical applications for PreDictor 96-well plates are shown in Table 1.

1.4 How PreDictor plates work and how screening experiments are performed

Each well in a PreDictor plate represents a batch. The main difference between a batch and a column is that in the batch, each separation only occurs once. In the column, we see a cascade of stages, sometimes referred to as theoretical plates.

The batch system can be used to investigate the distribution of target protein between the chromatography medium and the liquid phase. The batch experiment involves the same steps of equilibration, sample addition, wash, and elution as a column chromatographic separation.

Experimental setups using high-throughput PreDictor 96-well plates allow simultaneous testing of many different conditions (factors) such as pH, salt, etc. Experimental setups such as Design of Experiments (DoE), which uses statistics to identify and define factors having the greatest impact on the process or product, facilitate complex screening of conditions using PreDictor plates. Other experimental setups can, however, also be used.

In summary, applying high-throughput techniques means that future-generation processes can be developed quicker and more thoroughly.

Table 1. Studies that can be performed with PreDictor plates

Study	Factors	Comments
Binding conditions	Protein concentration, conditions (salt, pH, etc.), incubation time	Qualitative and quantitative analyses of capacity
Wash conditions	Protein concentration, conditions (salt, pH, additives, etc.)	Intermediate wash step(s) can improve purity of the next step, i.e., elution
Elution conditions	Conditions (salt, pH, additives, etc.)	Conditions for step elution and also gradient elution can be studied. Small systematic differences in conditions can be used to mimic a chromatography gradient elution.
Cleaning-in-Place (CIP)	Conditions (salt, pH, additives, etc.), time	Effectiveness of different CIP solutions can be tested
Adsorption isotherms	Conditions (salt, pH, additives, etc.)	Utilizes the convenience of the 96-well plate format for constructing adsorption isotherms Determination of optimum phase ratio for capacity/wash/elution studies

02

Batch experiments using PreDictor plates

2.1 Batch experiments using PreDictor plates vs column chromatography

In a typical adsorption process, both the mass transfer mechanism (responsible for protein transport) and ligand selectivity are independent of the mode of operation, that is, the adsorption process is the same whether it occurs in a batch system or in a packed column. In a column however, the adsorption process occurs continuously during the transport of protein through the column. This repeated adsorption process is described as a cascade of stages (column theoretical plates) where adsorption occurs. One well in a PreDictor 96-well plate can be regarded as a single stage in such a cascade.

In a chromatography column, any separation taking place in a single stage is further magnified by the next stage in series. However, as long as a difference in adsorption capacities/rates for different constituents of a sample can be quantitated in a single well, the results obtained using PreDictor plates can be used to describe the same separation occurring in a column. The workflow in a batch experiment comprises the same steps as any column experiment — equilibration of the medium with the desired buffer, sample application, wash, and elution (Fig 2) — and therefore the same types of study can be performed on both formats (Table 1).

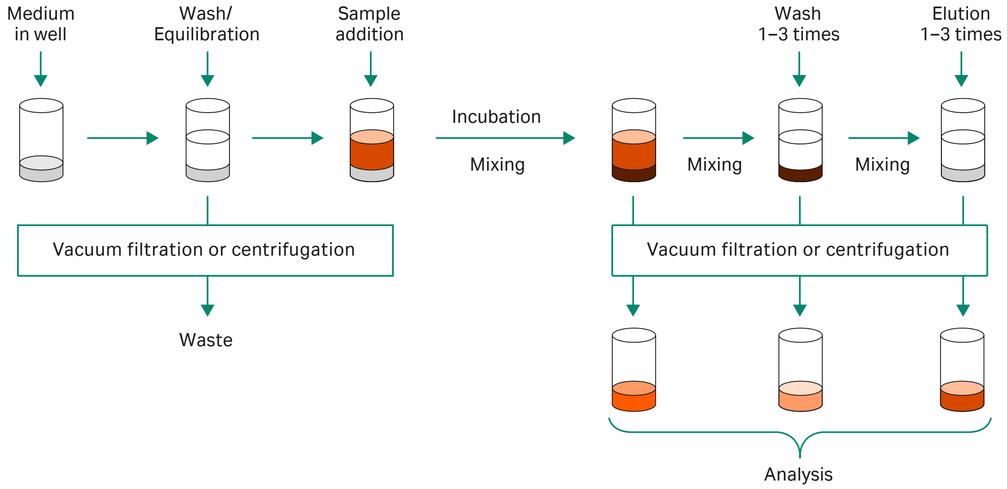


Fig 2. Schematic illustration of the workflow of a batch experiment in the wells of a PreDictor plate. The same steps would be employed in a column experiment, i.e., equilibration, sample addition, wash, and elution. The gray color in the wells is chromatography medium, orange shades describe different concentrations of protein solution, brown is medium with bound sample.

2.2 The batch experiment

Batch experiments investigate the distribution of target protein(s) between the solid phase (chromatography medium) and the liquid phase. In a finite bath (batch) system, the amount of target protein is constant and is either free in solution or bound to the medium in the well. Initially when sample is added, none of the target protein is bound to the medium. After a certain incubation time, however, it becomes distributed between the liquid and solid phases (Fig 3).

Two different approaches can be used to determine the distribution of a protein between the phases:

1. Calculate the amount of protein bound to the solid phase (m_{bound}) as the difference between the amount added to the well (m_{added}) and the amount remaining in the liquid phase ($m_{unbound}$):

$$m_{bound} = m_{added} - m_{unbound} \quad (1)$$

2. Perform a number of consecutive wash and elution steps to elute the protein. The amount of eluted protein (m_{eluate}) is the amount of protein bound (m_{bound}) to the solid phase:

$$m_{bound} = m_{eluate} \quad (2)$$

When performing a full experiment with all steps, the full mass balance can be obtained by collecting and analyzing all fractions (flowthrough [FT], wash, elution). The amount added should be found either as unbound or bound protein:

$$m_{added} = m_{bound} + m_{unbound} \leftrightarrow m_{added} = m_{elaute} + m_{FT} + m_{wash} \quad (3)$$

The recovery can be calculated from the amount bound, unbound, and added:

$$\text{Recovery (\%)} = 100 \times \frac{m_{bound} = m_{unbound}}{m_{added}} \quad (4)$$

Yield can be calculated from the amount of protein eluted from the solid phase and the amount bound:

$$\text{Yield (\%)} = 100 \times \frac{m_{eluate}}{m_{bound}} \quad (5)$$

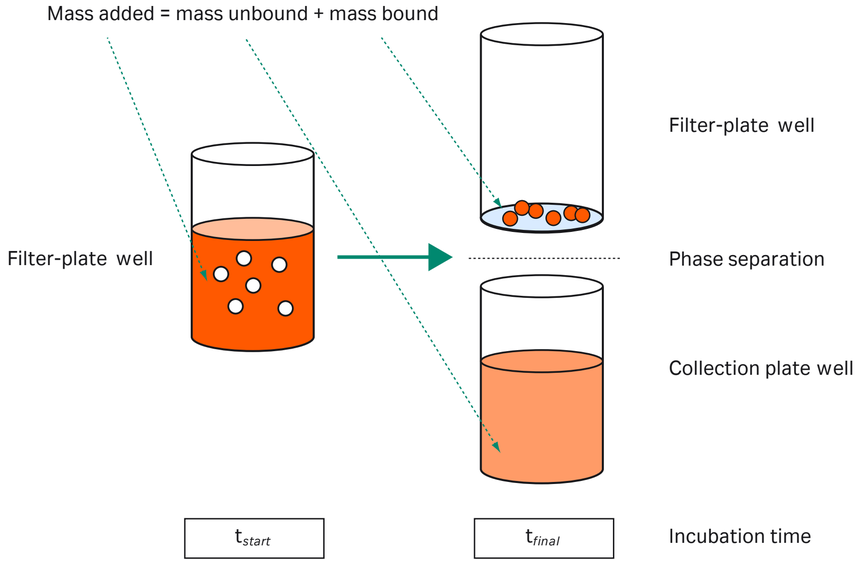


Fig 3. Schematic illustration of the mass balance in a single well. At t_{start}, all protein is in liquid phase. At t_{final}, the protein is distributed between the solid and liquid phases.

2.3 Capacity determinations

The two different approaches described above for determining the amount of protein bound to the medium can be used to calculate the capacity of a medium under a given set of experimental conditions.

Equation 1 can also be expressed in terms of concentration (Eq. 6):

$$m_{bound} = m_{added} - m_{unbound} \leftrightarrow V_{medium} \times q = V_{liq} \times (c_o - c_{unbound}) \quad (6)$$

where q is the binding capacity of the medium under given conditions at termination of incubation, V_{medium} is the volume of medium in the well, V_{liq} is the volume of liquid in the well, c_o is the concentration of target protein at the start of the experiment (t=0), and $c_{unbound}$ is the concentration of target protein found in the flowthrough (liquid phase) at termination of incubation.

Binding capacity can also be calculated from the protein concentrations found in the eluate(s):

$$q = \frac{\sum_{1}^{i} V_{eluate,\,i} \times C_{eluate,\,i}}{V_{medium}} \quad (7)$$

where i = each individual elution operation

Always calculate capacities using flowthrough data unless the experimental conditions are such that all of the bound material is recovered in the eluate fractions.

For most accurate results in the calculations, the volume of retained liquid in a well (V_r) has to be accounted for. Vacuum filtration and centrifugation do not entirely eliminate all liquid from PreDictor plate wells; liquid always remains both in the pores of the chromatography medium and in the filters of the plate wells after these procedures. With PreDictor plates, measurements have shown that the volume of liquid retained by the medium is approximately 60% of the medium volume (V_{medium}), and that about 6 µL of liquid is retained in the filter. The retained liquid volume, V_r is therefore a function of the medium volume (V_{medium}) in the well:

$$V_r = 6\ \mu L + 0.6\ V_{medium} \quad (8)$$

When accounting for retained volume in, for example, Equation 6, use

$$C_o = \frac{C_{sample} \times V_{sample}}{V_{sample} + V_r} \quad (9)$$

instead of

$$C_o = C_{sample} \quad (10)$$

and

$$V_{liq} = V_{sample} + V_r \quad (11)$$

instead of

$$V_{liq} = V_{sample} \quad (12)$$

2.4 The adsorption isotherm

A good understanding of thermodynamic and kinetic effects is needed to correctly describe a chromatographic separation. The thermodynamics of protein adsorption is described by the adsorption isotherm, which describes the relationship between concentration of protein in the liquid and solid phases at equilibrium under a given set of experimental conditions. Among many types of adsorption isotherms, the Langmuir isotherm is frequently used to describe protein adsorption. It is the only type of isotherm discussed in this handbook. Equation 13 describes the Langmuir isotherm for a single-component system.

$$q = \frac{q_{max} \times c_{eq}}{K_d + c_{eq}} \qquad (13)$$

where q_{max} is the maximum saturation capacity, K_d is the equilibrium dissociation constant, c_{eg} is the concentration of target protein in the liquid phase at equilibrium, and q is the binding capacity of the medium (concentration of target protein in the solid phase). The isotherm thus describes how q changes with c_{eq} as illustrated in Figure 4.

The isotherm provides information about the maximum capacity (q_{max}) of the medium for a given target molecule under given conditions. It also provides information on the binding strength (K_d). With good binding conditions (low K_d), the isotherm is relatively "rectangular", that is, it has a large plateau in which the capacity is relatively independent of the equilibrium concentration. The linear part of the isotherm is reached at low equilibrium concentrations. In this region, capacity is proportional to the equilibrium concentration (Fig 4).

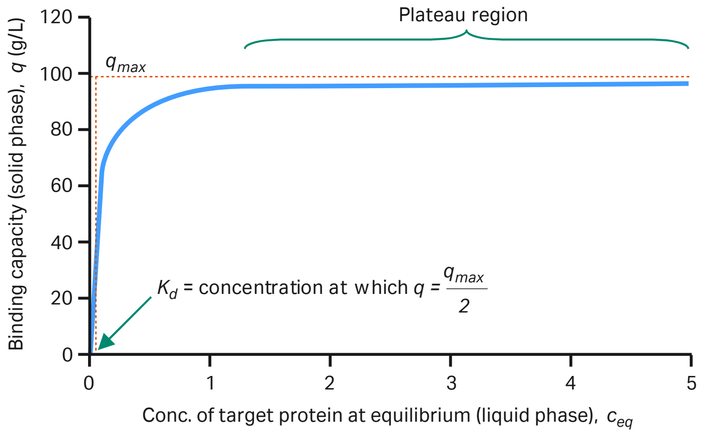


Fig 4. A Langmuir isotherm where the maximum binding capacity, q_{max} = 100 g/L and dissociation constant, K_d = 0.1 g/L are both indicated by red lines. The plateau region where capacity is relatively independent of equilibrium concentration is indicated.

2.5 Incubation time

Incubation time (also known as contact time) describes how long the target protein is in contact with the medium. Note that capacity varies with incubation time. For long incubation times, capacity will approach the equilibrium dictated by the isotherm (Fig 4). For shorter times, it will depend on the kinetics of uptake (Fig 5). If these kinetics are fast, the capacity difference between the two incubation times will be small. If slow, this difference will be large.

If plate incubation times are compared with column residence times, incubation times are generally longer. This difference relates to differences in the techniques. Incubation time corresponds better to the total loading time in columns since it reflects the total time chromatography media beads are in contact with the sample.

- An incubation time of 60 min in PreDictor plate experiments will generally give a good estimate of the potential binding capacity. Shorter times may suffice, depending on the uptake kinetics.
- For adsorption isotherm studies, longer incubation times (e.g., 3 h) are preferable. Some systems will nevertheless give an adequate estimation of the isotherm with an incubation time of just 60 min.

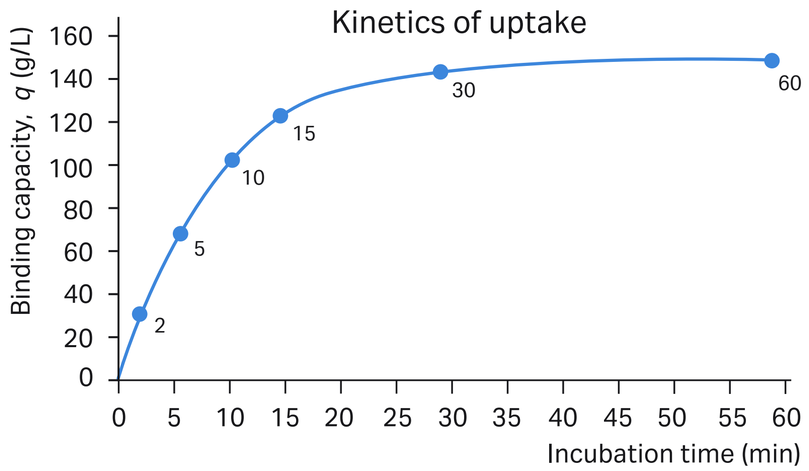


Fig 5. Curve demonstrating the kinetics of uptake, with binding capacity as a function of incubation time (2, 5, 10, 15, 30, and 60 min).

2.6 Phase ratio

An important aspect to consider when setting up an experiment is the phase ratio (β), which is the ratio between liquid volume and media volume. The mass balance equation (Eq. 6) can be rewritten with the phase ratio.

$$q = \beta c_o - \beta c$$

where

$$\beta = \frac{V_{liq}}{V_{medium}} \qquad (14)$$

For a given system, β and c_o are constants, and q is therefore a linear function of the concentration of target protein in the liquid phase, c. The line described by the equation is called the operating line (Fig 6). Any measurement with the given system will be found along this line.

The operating line starts at $c = c_o$ (time 0) and has a slope that equals - β. During the experiment, the capacity will increase along this line. For short incubations, the capacity obtained will be located far down on the operating line, while for very long incubations (where equilibrium is approached), the capacity will approach the capacity dictated by the isotherm. If the phase ratio is low (i.e., large volume of medium per well or small liquid volume), the potential capacity of the medium is not being explored, because the intercept between the operating line and the underlying isotherm is not in the plateau region of the isotherm. In simple terms, there is too much medium to reach the maximum capacity of the medium, q_{max}.

1. The type of study will determine the medium volume to use.
2. Use small volumes for capacity studies.
3. Use larger volumes for wash and elution studies.

Several different types of PreDictor plates have been developed for different applications. To select the relevant plate type, see Section 7.

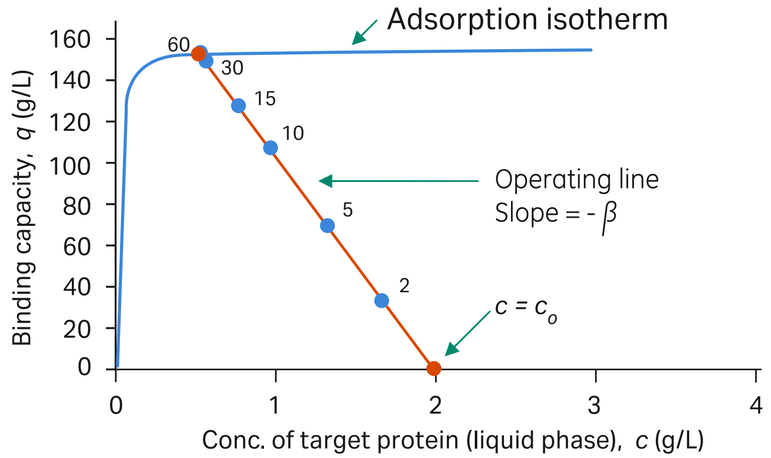


Fig 6. A graphical illustration of capacity vs concentration. The adsorption isotherm, the operating line and incubation times along the operating line are shown. The operating line (orange) is given by the experimental setup (V_{liq}, V_{medium}, c_o). The operating line starts at c=c_o and ends at q defined by the isotherm. The slope of the operating line is equal to the negative of the phase ratio in the system, $-\beta = -V_{liq}/V_{medium}$.

2.7 Sample concentration and media volume

The underlying adsorption isotherm strongly influences the capacity of the medium. For capacity studies, operations should take place in the plateau region of the isotherm, that is, where capacity is relatively independent of the concentration of target protein in the liquid phase. Set up the system so that the final concentration in the liquid phase corresponds to the capacities in the plateau region (refer to the operating line in Fig 6). Once the favorable binding conditions have been found, the isotherm is relatively rectangular; only at very low concentrations will the capacity be dramatically affected by the concentration.

For capacity experiments, ensure that an excess of target protein is added to the well so that the chromatography medium is overloaded (see the example in Section 2.9.1).

For wash and elution studies, a somewhat larger medium volume in the wells is often advantageous to ensure that sufficient amounts of protein and/or impurities for detection and analysis are obtained. When larger volumes are used, multiple loads can be required to reach the desired amount bound (see Section 4.3).

Understanding the effects of the phase ratio and the isotherm on capacity facilitates study planning.

2.8 Mixing

In batch experiments, agitation is essential. Without sufficient agitation, the rate-limiting mass transfer will be different in wells compared with a chromatography column. Many factors affect mixing efficiency, for example, well diameter, liquid volume, liquid viscosity/density, particle density, and orbital amplitude of the shaking table. Figure 7 illustrates the effects of liquid volume and agitation speed.

Use an agitation speed of 1100 rpm with a 3 mm circular centripetal movement and a liquid volume of 100 to 300 µL in the wells. Mix in all steps (equilibration, loading, wash, and elution)!

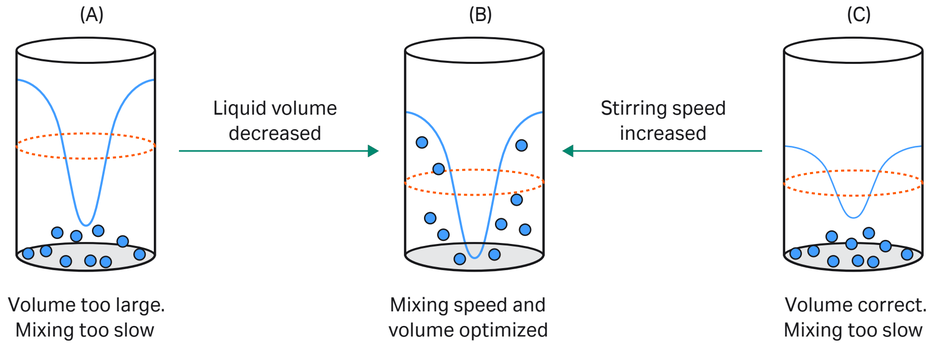


Fig 7. Schematic illustration of the effect of liquid volume and agitation speed on mixing efficiency. The situation before (orange) and during (blue) mixing is shown. (A) Liquid volume too large, (B) liquid volume and agitation speed optimized, (C) agitation speed too low.

2.9 Types of studies

The examples below refer to studies performed in PreDictor plates.

2.9.1 Binding studies

Binding studies can be performed in single-medium plates, screening plates, or adsorption isotherm plates (see Section 7). With single-medium plates, 96 wells per plate are available for the experiment, while with screening plates, 24 or 32 wells are available per chromatography medium and plate. If isotherm plates are used, the response per condition is a whole isotherm and not just a capacity value. With isotherm plates, a maximum of 16 different conditions can be studied per plate.

Use low-volume plates (2 or 6 µL) for binding studies in single-medium plates or screening plates. Low media volumes are easier to saturate, that is, load to maximum capacity.

When planning the binding study, make sure to load enough protein. At termination of the incubation, the sample concentration should not decrease to less than 50% of the initial concentration. This ensures working in the plateau region of the adsorption isotherm and achieves the highest precision in analysis and calculations.

Decreasing to 80% of initial concentration is, however, acceptable when still working in the plateau region of the adsorption isotherm. Here, capacity is relatively independent of equilibrium concentration. By rearranging Equation 6, the excess of protein used in the experiment can be calculated:

$$\frac{c}{c_o} = 1 - \frac{q \times V_{medium}}{V_{liq} \times c_o} = 1 - \frac{100\ g/L \times 2\ \mu L}{200\ \mu L \times 4\ g/L} = 0.75 \qquad (15)$$

assuming a binding capacity of 100 g/L, feed concentration of 4 g/L, 200 µL of liquid volume, and 2 µL of chromatography medium.

Use an incubation time of 60 min. Shorter times may suffice if the uptake kinetics are fast. See the application example in Section 4.1.

2.9.2 Binding studies — time-dependent

In the batch system, the chromatography medium is incubated for different periods of time in the presence of the feed/sample (Fig 8).

Studying dynamic binding capacity (DBC) as a function of process conditions (pH, conductivity, residence time, feed concentration, etc.) is typically one of the first investigations of downstream development. The aim is to optimize conditions with respect to DBC and robustness, yet the work, when performed using columns, is laborious and requires a substantial amount of valuable sample.

PreDictor plates can be used for time-dependent studies and as outlined in Figure 8, the chromatography medium is incubated for different periods of time in the presence of the feed/sample. Low-volume, single-medium plates (2 or 6 µL) work best (see *Plate selection guide*, Section 7.1).

Using PreDictor plates to investigate time-dependent protein uptake on chromatography media (Fig 8A) allows prediction of DBC data as a function of residence time in a chromatography column. Studies can either be quantitative or qualitative, that is, enable relative ranking of rate of uptake.

Qualitative study: Using different incubation times in the batch uptake system (e.g., 5 and 60 min) provides valuable information that can indicate the effect of residence time in a column, as schematically illustrated in Figure 9.

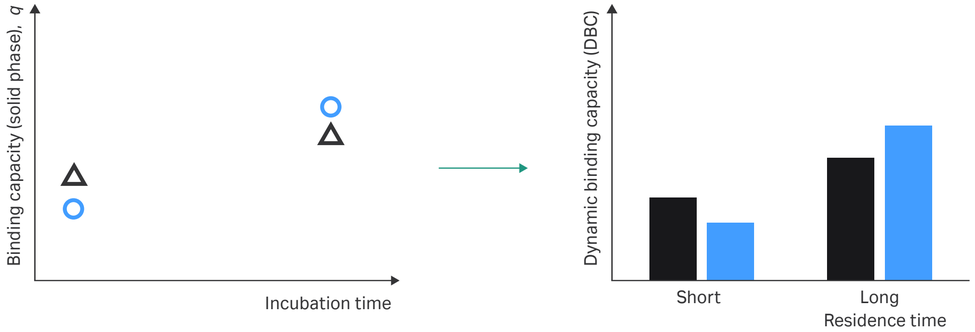
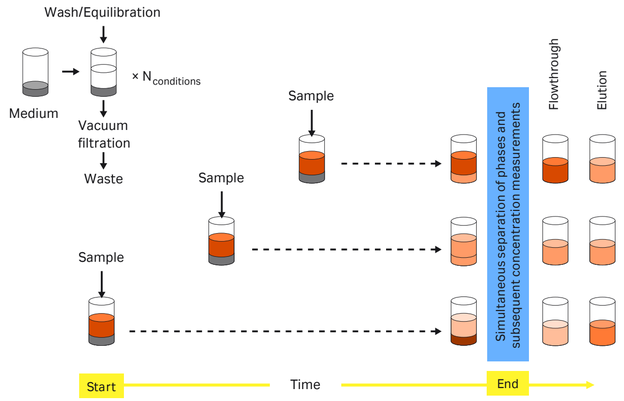


Fig 9. Batch experiments (left), performed with a short and a long incubation time, give qualitative information on trends that can be observed in column experiments (right).

(A)



(B)

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.5 min	2.5 min	5 min	5 min	10 min	10 min	15 min	15 min	30 min	30 min	60 min	60 min
B	2.5 min	2.5 min	5 min	5 min	10 min	10 min	15 min	15 min	30 min	30 min	60 min	60 min
C	2.5 min	2.5 min	5 min	5 min	10 min	10 min	15 min	15 min	30 min	30 min	60 min	60 min
D	2.5 min	2.5 min	5 min	5 min	10 min	10 min	15 min	15 min	30 min	30 min	60 min	60 min
E	2.5 min	2.5 min	5 min	5 min	10 min	10 min	15 min	15 min	30 min	30 min	60 min	60 min
F	2.5 min	2.5 min	5 min	5 min	10 min	10 min	15 min	15 min	30 min	30 min	60 min	60 min
G	2.5 min	2.5 min	5 min	5 min	10 min	10 min	15 min	15 min	30 min	30 min	60 min	60 min
H	2.5 min	2.5 min	5 min	5 min	10 min	10 min	15 min	15 min	30 min	30 min	60 min	60 min

Fig 8. (A) Outline of experimental protocol for the high-throughput batch uptake method with variable incubation time. (B) Example of a PreDictor 96-well plate layout that can be used. Four different conditions (indicated by green, yellow, orange, and blue shading) with six different incubation times (2.5, 5, 10, 15, 30, and 60 min) in quadruplicate are studied. Recommended incubation times (in minutes) are shown in each well.

Quantitative study: DBC versus residence time can be predicted based on data from a batch system, using a combination of modeling and experimental approaches (1–5).

The rate of uptake of the target protein (and other components if desired) onto the chromatography medium is measured and subsequently described in a mathematical model. A similar model is then used to describe protein adsorption in a chromatography column and the DBC versus residence time relationship is simulated. The method is schematically illustrated in Figure 10. An application example that predicts the DBC of human IgG on MabSelect SuRe™ is described in Reference 6.

In addition to determining DBC data, the method can be used to study the effect of incubation time on the purity of the target protein by monitoring changes in contaminant concentration with time.

2.9.3 Flowthrough studies (non-binding mode)

Studies of chromatographic steps using flowthrough mode are performed where, instead of maximizing (or optimizing) the binding of the target protein, the focus is on maximizing contaminant binding while maintaining a high yield of the target protein in the flowthrough fraction.

2.9.4 Wash and elution studies

Wash and elution studies are performed to optimize the purity and/or yield of the target protein. When the effect of load can influence the results, use similar loads in the plate and in the column experiments.

Based on the specific application, the desired column load (in terms of percent of DBC) should be defined. In PreDictor plates (batch mode), this load is obtained from binding experiments in low-volume plates (2 or 6 µL). Note that the phase ratio changes when scaling up to recommended plate volumes of 20 µL (first choice) or 50 µL (second choice) for wash and elution studies. Multiple loadings may be required to obtain the desired load with 20 or 50 µL plates as the maximum volume per load is 300 µL. An example using multiple loads is shown in Section 4.3. For some studies, the load is not necessarily critical. Section 4.4 shows such an elution study example.

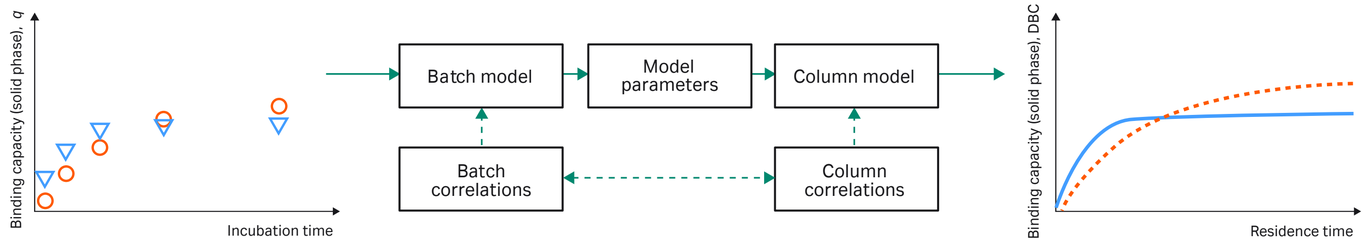


Fig 10. Outline of data and information flow for a quantitative method for determining the effect of residence time on DBC. The dashed line represents an optional method expansion. Batch experiments are shown on the left and the predicted DBC on the right.

2.9.5 Cleaning-in-Place (CIP) studies

Traditionally, small-scale columns are used for CIP studies. However, such studies are labor intensive and time — and sample — consuming. In addition, small-scale columns are limited to evaluation of one CIP condition at a time. By using PreDictor plates, several cleaning protocols can be evaluated in parallel. Section 4.5 shows the development of a CIP protocol for a protein A affinity medium in PreDictor plates.

2.9.6 Adsorption isotherm studies

Adsorption isotherms give information about binding strength (K_d) and potential binding capacity (q_{max}) under the conditions studied. With PreDictor plates, different approaches can be used to obtain adsorption isotherms. For example:

1. sample concentration is kept constant while the phase ratio is varied,
2. phase ratio is kept constant and the sample concentration is varied, and
3. combination of approaches 1 and 2.

The use of adsorption isotherm plates (Approach 1) simplifies the experimental protocol as no sample manipulation is necessary. The adsorption isotherm plates contain variable amounts of medium in the wells (Fig 11A). The same sample (same concentration and volume) is applied to all wells. Approach 2 uses single-volume plates and the initial concentration of sample is varied while the phase ratio is kept constant (Fig 11B). Thus, if single-volume plates are used, dilution of the sample is necessary in order to obtain different initial concentrations. For both approaches, varying the sample volume added to the wells is also possible. Note, however, that as the recommended V_{sample} is limited to between 100 and 300 µL, this technique must be used in combination with Approach 1 or 2 in order to cover the whole isotherm. An application example is given in Section 4.6.

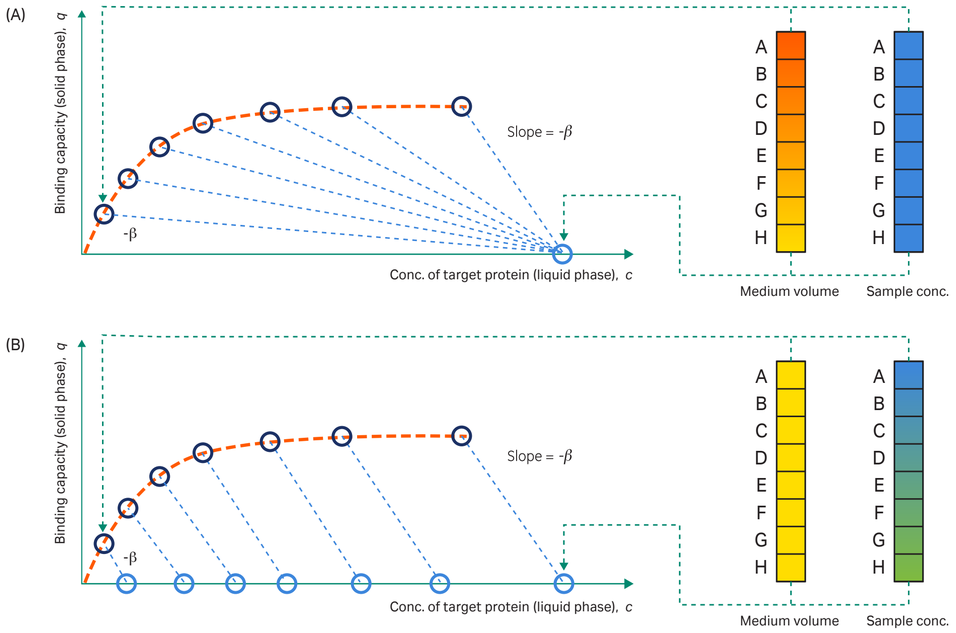


Fig 11. Illustration of two methods to determine adsorption isotherms in a batch system. (A) Constant initial concentration (c_o) and varying phase ratio (β), (B) Constant β and varied initial concentration, c_o. Colored bars show variables and constants in the different methods to determine adsorption isotherms (see text for details).

2.9.7 Chromatography media screening

PreDictor plates can be used to compare different chromatography media. PreDictor anion exchange (AIEX) and cation exchange (CIEX) media screening plates are available (see *PreDictor plate selection*, Section 7). Each type of screening plate is available either in low-volume (2 µL/6 µL) or high-volume formats (20 µL). The workflow used is the same as with the single-medium plates, except that 24 or 32 wells are available for each medium instead of 96 (Fig 12).

Note that a multimodal ion exchanger (Capto adhere and Capto MMC) is included in each type of plate. Because of their special properties (7, 8), Capto adhere and Capto MMC can have a different optimal operation window than traditional ion exchangers. Capto adhere, for example, has been shown to bind the target protein at pH values significantly below the isoelectric point (Fig 13). The optimal conductivity can also differ from that of traditional ion exchangers.

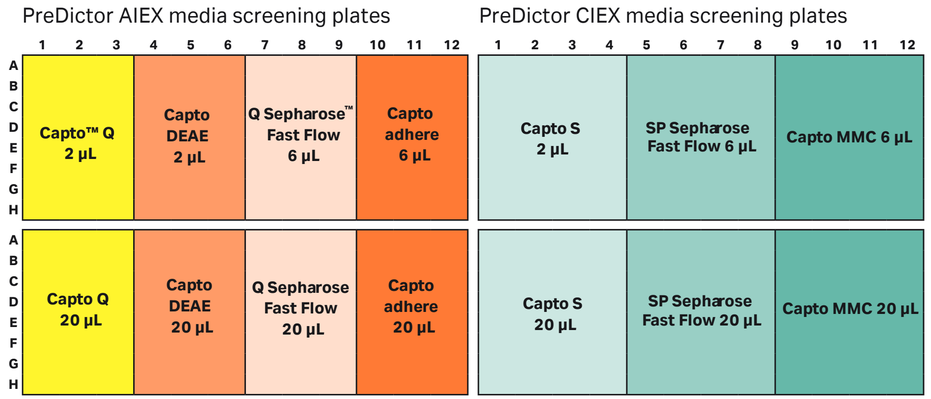


Fig 12. Plate layout of PreDictor AIEX and CIEX media screening plates. Low-volume plates contain 2 µL of medium/well (high-capacity Capto media) and 6 µL of medium/well (other media). High-volume plates contain 20 µL of medium/well for each of the media shown.

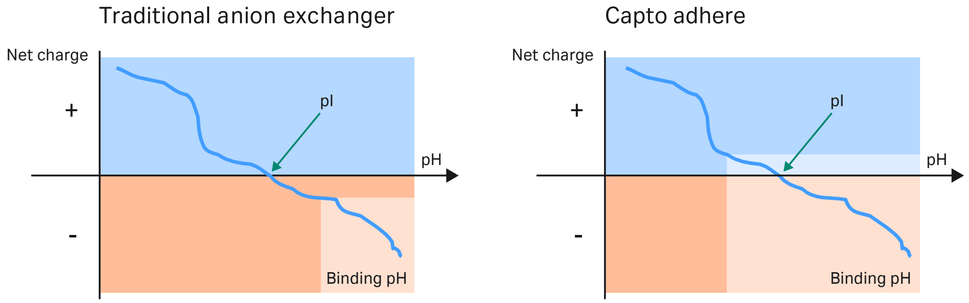


Fig 13. Schematic illustration of a titration curve for a protein. Different binding pH should be expected on traditional ion exchangers and Capto adhere. In most cases, Capto adhere will bind the protein at a pH significantly below the isoelectric point (pI).

03

Practical considerations

Sample prerequisite: Samples should be clarified prior to application using normal-flow filtration since unclarified samples can clog the wells.

Analytics: The precision and sensitivity of the analytical method used can influence what can be achieved with a given experimental setup.

Capacities can, for example, be calculated using flowthrough data or from elution data, as discussed in Section 2.3. If the amount bound is measured via the mass balance equation (Eq. 6), the analytical method must quantitate $\Delta c = c_o - c$. If the amount bound is measured from eluted fraction(s), the concentration in the eluate fractions must be quantifiable. A requirement for estimating capacity from eluted material is that close to 100% of the bound amount is eluted with the chosen elution conditions. For further discussion on the quality of data, see Section 5.

The 96-well plate format allows a large number of conditions to be studied in parallel, which puts pressure on the analytical methods used and resources available. With this in mind, a sequential approach to the experimental setup can be considered. This can be a funnel approach, where the initial broad screening is performed on responses with rapid analytical methods. From this initial screening, conditions can be selected for responses that require more advanced (or laborious) analytical methods.

Incubation time vs residence time: Incubation time (contact time) is not quantitatively comparable to residence time in columns. Incubation time better corresponds to the total loading time in columns since this reflects the total time the medium particles are in contact with the sample. If long incubation times are used, the measured capacity better reflects the equilibrium capacity of the medium. For short incubation times, the rate of mass transfer will affect the measured capacity (Fig 5).

Pipetting, buffer preparation, and sample preparation: Buffer and sample preparation can be relatively time-consuming with a manual workflow. If a robotic system is available, buffer preparation can be set up on the robot, thus simplifying this labor-intensive activity. Pipetting should be performed with multipipettes (8- or 12-tip) or by a robot. For practical transfer in a reasonable time, buffers and samples should be prepared in and dispensed from deep-well plates (4, 8, 12, 48 wells) according to the experimental plate layout.

Minimizing leakage from the plate during operation: To minimize risk of leakage through the bottom filter, it is important to avoid direct contact between the PreDictor plate outlets (the drips on the bottom) and any surface. Repeated loadings and long incubation times using buffers or samples containing certain detergents can induce leakage through the filter. Recommendations for minimizing leakage when working with detergents are provided in the PreDictor plate Instructions (*Instructions* 28925834).

Mixing during incubation: Adequate mixing must be used during all incubation steps in order to keep the chromatographic medium suspended in the liquid phase (see Section 2.8 and Reference 6).

UV as quantitation method: If UV absorbance is used to determine concentrations, ensure that the calibration curve is linear and covers the range of concentrations to be measured; extrapolation is not recommended. Use standard procedures to compensate for potential light scattering effects, blank absorbance, and pathlength.

Transferring experiments from low- to high-medium volume plates: When transferring experiments from low-volume plates to high-volume plates, the capacity obtained on the high-volume plate can be much lower than on the low-volume plate. The reason for this is the different phase ratios obtained with the different plates containing different media volumes. Multiple loading will possibly have to be performed in order to reach the desired load (Fig 17).

Evaporation and sealant: When longer incubation times are used (more than 60 min) as in, for example, adsorption isotherm studies, consider sealing the plates to prevent evaporation. Cytiva microplate foils (96 well), code number BR100578 can be used. These microplate foils are transparent, self-adhesive plastic films where the adhesive does not cover the well. (avoids contact between the sample and the adhesive).

Liquid compatibility: PreDictor plates and the chromatography media with which they are prefilled are compatible with all solutions commonly used in the purification of biopharmaceuticals, see Instructions 28925834 for details.

Plate re-use: PreDictor plates are disposable items. Repeated use can cause leakage as well as cross-contamination between experiments.

04
Application examples

4.1 Screening binding conditions on Capto S using PreDictor plates

This application presents a binding study performed with a 60 min incubation. The binding capacity of Capto S for two proteins, conalbumin and α-chymotrypsin, was investigated at different ionic strengths and pH. The data obtained were compared to results obtained with column experiments (9).

Experimental: PreDictor plate

Plate: PreDictor Capto S, 2 µL

Sample: 3.5 g/L conalbumin or α-chymotrypsin in each equilibration buffer

Equilibration/loading buffers: Fifteen different buffers comprising 20 mM sodium acetate at three pH levels (4.25, 4.75, 5.25) supplemented with sodium chloride to obtain the desired ionic strengths (0.02, 0.05, 0.08, 0.12, 0.15)

Steps:

Equilibration 1–3: 200 µL equilibration buffer, 1 min incubation

Sample loading: 200 µL sample solution, 60 min incubation

Mixing was performed on an orbital shaker at 1100 rpm during all incubation steps. Liquids were removed by vacuum filtration.

The concentration of unbound protein was quantitated in the flowthrough from the loading step by reading UV absorbance. The amount bound was determined from the mass balance equation (Eq. 6). Experimental design and plate layout is shown in Figure 14.

Experimental: Column chromatography

Column: 2 mL of Capto S packed in a Tricorn™ 5/100 column

Sample: Approx. 4 g/L conalbumin or α-chymotrypsin in each equilibration buffer

Equilibration/loading buffers: 15 different buffers. 20 mM sodium acetate at three pH levels (4.25, 4.75, 5.25) supplemented with sodium chloride to obtain the desired ionic strengths (0.02, 0.05, 0.08, 0.12, 0.15)

Residence time: 2 min

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.02 4.25 Conalb	0.02 4.25 Conalb	0.02 4.25 Conalb	0.118 4.75 Conalb	0.118 4.75 Conalb	0.118 4.75 Conalb	0.02 4.25 Chymo	0.02 4.25 Chymo	0.02 4.25 Chymo	0.118 4.75 Chymo	0.118 4.75 Chymo	0.118 4.75 Chymo
B	0.053 4.25 Conalb	0.053 4.25 Conalb	0.053 4.25 Conalb	0.15 4.75 Conalb	0.15 4.75 Conalb	0.15 4.75 Conalb	0.053 4.25 Chymo	0.053 4.25 Chymo	0.053 4.25 Chymo	0.15 4.75 Chymo	0.15 4.75 Chymo	0.15 4.75 Chymo
C	0.085 4.25 Conalb	0.085 4.25 Conalb	0.085 4.25 Conalb	0.02 5.25 Conalb	0.02 5.25 Conalb	0.02 5.25 Conalb	0.085 4.25 Chymo	0.085 4.25 Chymo	0.085 4.25 Chymo	0.02 5.25 Chymo	0.02 5.25 Chymo	0.02 5.25 Chymo
D	0.118 4.25 Conalb	0.118 4.25 Conalb	0.118 4.25 Conalb	0.053 5.25 Conalb	0.053 5.25 Conalb	0.053 5.25 Conalb	0.118 4.25 Chymo	0.118 4.25 Chymo	0.118 4.25 Chymo	0.053 5.25 Chymo	0.053 5.25 Chymo	0.053 5.25 Chymo
E	0.15 4.25 Conalb	0.15 4.25 Conalb	0.15 4.25 Conalb	0.085 5.25 Conalb	0.085 5.25 Conalb	0.085 5.25 Conalb	0.15 4.25 Chymo	0.15 4.25 Chymo	0.15 4.25 Chymo	0.085 5.25 Chymo	0.085 5.25 Chymo	0.085 5.25 Chymo
F	0.02 4.75 Conalb	0.02 4.75 Conalb	0.02 4.75 Conalb	0.118 5.25 Conalb	0.118 5.25 Conalb	0.118 5.25 Conalb	0.02 4.75 Chymo	0.02 4.75 Chymo	0.02 4.75 Chymo	0.118 5.25 Chymo	0.118 5.25 Chymo	0.118 5.25 Chymo
G	0.053 4.75 Conalb	0.053 4.75 Conalb	0.053 4.75 Conalb	0.15 5.25 Conalb	0.15 5.25 Conalb	0.15 5.25 Conalb	0.053 4.75 Chymo	0.053 4.75 Chymo	0.053 4.75 Chymo	0.15 5.25 Chymo	0.15 5.25 Chymo	0.15 5.25 Chymo
H	0.085 4.75 Conalb	0.085 4.75 Conalb	0.085 4.75 Conalb				0.085 4.75 Chymo	0.085 4.75 Chymo	0.085 4.75 Chymo			

Conalbumin | **α-Chymotrypsin**

Fig 14. Plate layout for the experiment described in section 4.1. The 15 different conditions are shown in different colors. Six wells (empty cells) were not used.

By recording the UV-signal during sample load the dynamic binding capacity at 10% breakthrough was calculated.

Results and discussion

The two proteins behave differently with respect to ionic strength. α-Chymotrypsin behaves in a traditional manner with the highest capacity obtained at low conductivity (Fig 15A and C). Conalbumin, on the other hand, behaves in a non-traditional manner (Fig 15B and D) (9, 10, 11). The trends observed with PreDictor plates (Fig 15A and B) are essentially the same as those obtained in column experiments (Fig 15C and D). Binding capacities in plates obtained with a 60 min incubation correspond very well to dynamic binding capacities obtained in columns with a residence time of 2 min. This is because incubation time better corresponds to the total loading time in columns since it reflects the total time the media particles are in contact with the sample.

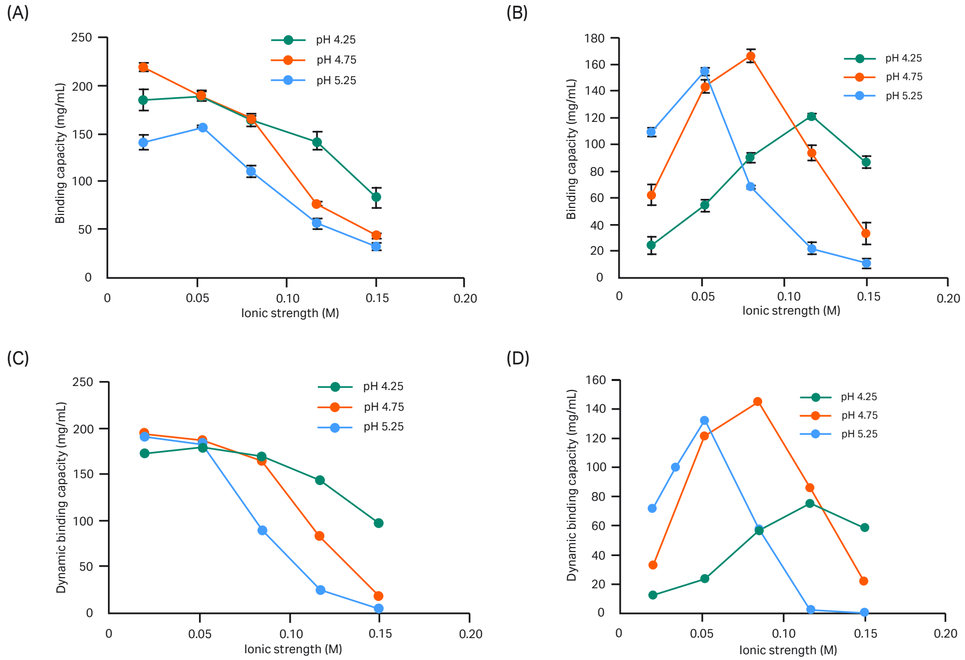


Fig 15. Determination of loading conditions for α-chymotrypsin and conalbumin on Capto S. Binding capacities of α-chymotrypsin (A) and conalbumin(B) after 60 min incubation in PreDictor Capto S, 2 µL plates. Dynamic binding capacities at 10% breakthrough for α-chymotrypsin (C) and conalbumin (D). The residence time was 2 min and the column was a Tricorn 5/100 (CV 2 mL).

4.2 Effect of incubation time, pH, and ionic strength on the binding of amyloglucosidase to Capto DEAE

Taking a time-dependent study all the way from plate data to predicted dynamic binding capacity (DBC) values in a column is not always necessary. As shown below, valuable qualitative information can be obtained from an incubation time study without predicting DBC. An incubation time of 60 min is generally recommended for capacity studies. Shorter and longer incubation times provide more data on how optimal conditions change over time.

Experimental: PreDictor plate

Plate:	PreDictor Capto DEAE, 2 µL
Sample:	4 g/L amyloglycosidase in each equilibration buffer
Equilibration/loading buffers:	30 mM buffer, Tris pH 8 and 9, or bis-Tris pH 6 and 7, and 12 levels of total ionic strengths between 0.02 and 0.5 M at each pH. Sodium chloride was used to obtain the desired ionic strengths

Steps:

Equilibration 1–3:	200 µL equilibration buffer, 1 min incubation
Sample loading:	150 µL sample solution at three different incubation times; 2 min, 60 min, or 20 h.

Mixing was performed on an orbital shaker at 1100 rpm during all incubation steps. Liquids were removed by vacuum filtration at -300 mbar for 5 s during all steps. The filtrate fraction from the loading was collected in a UV-readable multiwell plate and absorbance at 280 nm was read. The absorbance was used to calculate protein concentration by applying a standard curve prepared using protein stock solutions. Binding capacities were calculated from the mass balance equation (Eq. 6).

Results and discussion

Contour maps describing the effects of ionic strength, pH, and incubation time on capacity were generated directly from raw data (i.e., no modeling or fitting was involved). The contour maps show how optimal conditions for capacity vary with incubation time.

In this study, the pH optimum (pH 7) was the same for all incubation times, but the optimal ionic strength varied from 0.07 at 2 min, through 0.04 at 60 min, to 0.02 at 20 h (Fig 16). This is an important result from a process development perspective as the shift in optimal conditions observed can be expected as an effect of column residence time. The maps shown in Figure 16, generated from SigmaPlot™ v8.0 software (Systat Software Inc.), are characteristic for ion exchange situations where changes in ionic strength and pH have opposite effects on intraparticle mass transfer rates and on equilibrium capacity. The reduced equilibrium capacity caused by an increase in ionic strength is balanced by improved intraparticle mass transfer resulting in a binding capacity maximum at intermediate ionic strength. The equilibrium capacity effect dominates at longer incubation times, shifting the maximum to lower ionic strengths. This type of non-traditional ion exchange behavior (9, 10, 11) can also be seen for conalbumin in Figure 15. For further details of this study, see Reference 6.

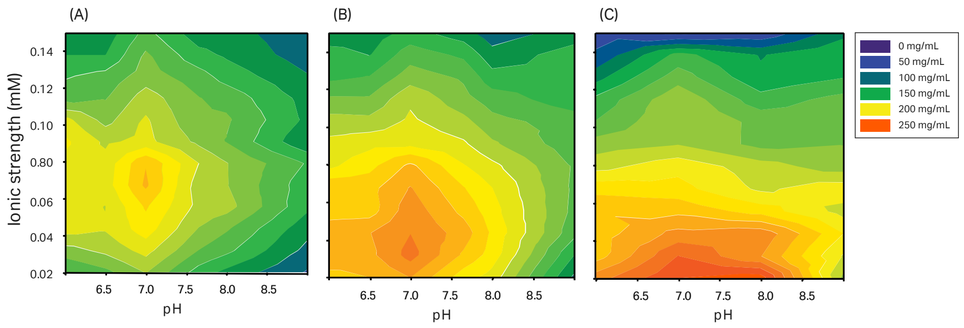


Fig 16. Contour maps showing the effect of ionic strength and pH on binding capacity of Capto DEAE for amyloglucosidase over (A) 2 min, (B) 60 min, and (C) 20 h. PreDictor plates filled with 2 µL of chromatography medium per well were used for the experiment.

4.3 Optimization of the wash step for MabSelect SuRe

The possibility of reducing host cell protein (HCP) levels in the elution pool from a protein A chromatographic step by introducing an intermediate wash step was investigated. The effect of 17 different intermediate wash buffers (variations in pH, NaCl concentration, and *n*-propanol concentration), as well as the effect on MAb yield was evaluated. The study was performed both in PreDictor plates and by column chromatography to check the correlation between the results obtained with the two formats.

Experimental: PreDictor plate

Plate:	PreDictor MabSelect SuRe, 50 µL
Sample:	MAb feed with a MAb concentration of 1.3 g/L
Equilibration buffer:	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Intermediate wash buffers:	17 different buffers (variations in pH, NaCl and *n*-propanol)
Elution buffer:	20 mM sodium citrate, pH 3.6

Steps:

Equilibration 1–3:	300 µL equilibration buffer, 1 min incubation
Sample loading[1]*:*	To reach a capacity of 23 g/L as used in the chromatography step described later, multiple loadings were needed; 3 × 300 µL of MAb feed was loaded each time and incubated for 20 min
Wash 1 and 2:	200 µL equilibration buffer, 1 min incubation
Wash 3 and 4:	200 µL intermediate wash buffer, 1 min incubation
Wash 5 and 6:	As wash 1 and 2, 1 min incubation
Elution 1–3:	200 µL elution buffer. The medium was incubated with elution buffer for 2 min before drainage. The three consecutive eluates were collected into three different UV-readable plates for analysis.

Liquid was removed by centrifugation and incubation was performed on a shaker (1100 rpm) in all steps.

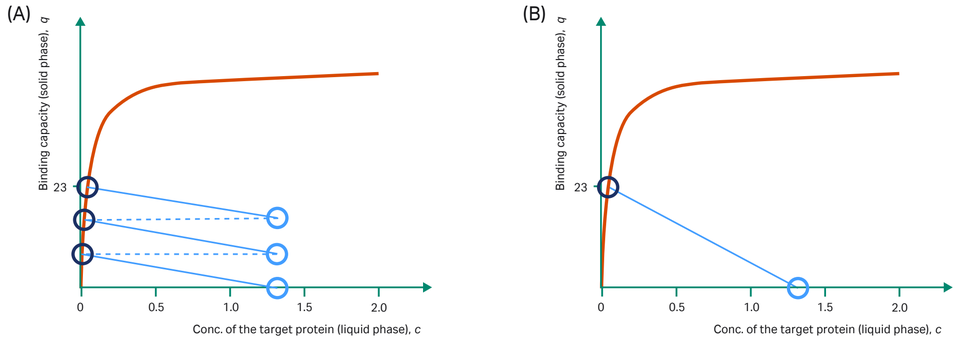


Fig 17. Schematic illustration of how to reach the desired load of 23 g/L. Multiple loadings on a 50 µL PreDictor plate (A) or a single load on a 20 µL plate (B). Circles represent the initial starting point (light blue) and final point (dark blue) for each incubation.

MAb concentration (UV absorbance) and HCP concentration (ELISA) were analyzed in the eluates.

[1] **Multiple loadings:** As discussed above, the underlying isotherm and phase ratio control the capacity achieved. In this application, an incubation time of 20 min was enough for the capacity to approach the isotherm capacity. Because of the relatively low phase ratio (6 = 300/50) and the relatively low feed concentration of the MAb, only about 1/3 of the desired load was reached by loading once. This was overcome by loading three times to reach a capacity or load of 23 g/L (Fig 17A). In this particular experiment, it would have been better to use a 20 µL PreDictor plate. If such a plate was used, the phase ratio would have increased from 6 to 15 (300/20) and the desired load (capacity) would have been attained in only one load (Fig 17B). Other options to reach the desired load are to change the phase ratio by using a different liquid phase volume (V_{liq}), and/or using a different initial concentration, C_o .

Experimental: Column chromatography

Column: HiTrap MabSelect SuRe, 1 mL

The steps employed are described in detail here:

Step	Column volume (CV)	Flow (cm/h)	Description
Equilibration	10	250	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Sample load	18	63	23 g/L medium (18 mL 1.3 g/L clarified MAb feed). 2.4 min residence time
Wash	6	250	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Intermediate wash	2	250	Intermediate wash buffer
Wash	2	250	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Elution	—	100	Fractionation with watch function based on UV levels (fractionation started at UV = 500 mAU and stopped at UV = 400 mAU)
Regeneration	4	250	100 mM sodium citrate, pH 3.0
CIP	10	100	0.5 M NaOH, 15 min contact time
Re-equilibration	10	250	20 mM sodium phosphate, 150 mM NaCl, pH 7.4

MAb concentration (UV absorbance) and HCP concentration (ELISA) were analyzed in the collected eluate.

Results and discussion

Compared with the reference run using a standard wash in a column, none of the intermediate washes improved the wash procedure with respect to HCP levels in the eluate fractions (Fig 18). Four of the intermediate wash buffers (expts. 1, 2, 10, and 11 in Fig 18) even proved detrimental in the column experiments as HCP levels higher than the reference were obtained. The plate experiments gave the same results except with wash buffer 2, where a detrimental effect was not observed. With respect to yield, some of the intermediate washes (expts. 2, 7, 14, and 16 in Fig 18) proved detrimental, while for others, yield was in the same range as the reference runs. Correlation between the yields obtained with the two methods was good.

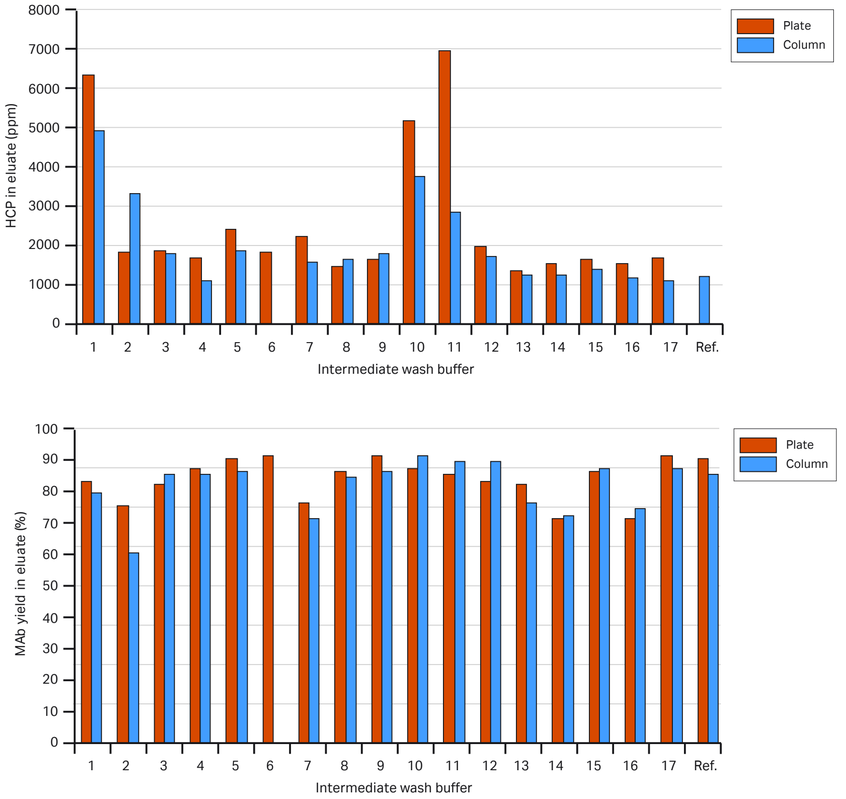


Fig 18. Effect of different wash buffers on the host cell protein (HCP) levels and monoclonal antibody yield in the elution pool from MabSelect SuRe. Results from plate experiments using PreDictor MabSelect SuRe, 50 µL (orange) are compared with column experiments using HiTrap MabSelect SuRe, 1 mL (blue).

4.4 Screening elution pH on MabSelect SuRe

A PreDictor MabSelect SuRe 50 µL plate was used to screen elution pH for five monoclonal antibodies. A simulated pH gradient was set up in the plates and the yield at different pH was measured. Results obtained with PreDictor plates were compared with results obtained with pH gradient elution in a Tricorn 5/100 column packed with 2 mL of MabSelect SuRe.

Experimental: PreDictor plate

Plate:	PreDictor MabSelect SuRe, 50 µL
Equilibration buffer:	20 mM sodium phosphate, 150 M NaCl, pH 7.4
Final wash buffer:	20 mM sodium citrate, pH 6.0
Elution buffer:	20 mM sodium citrate, pH 5.8–3.0
Samples:	1.3 g/L of five different MAb's, MAb no. 1, 3, 4, and 5 in equilbration buffer, MAb no. 2 in unpurified clarified NSO-cell supernatant

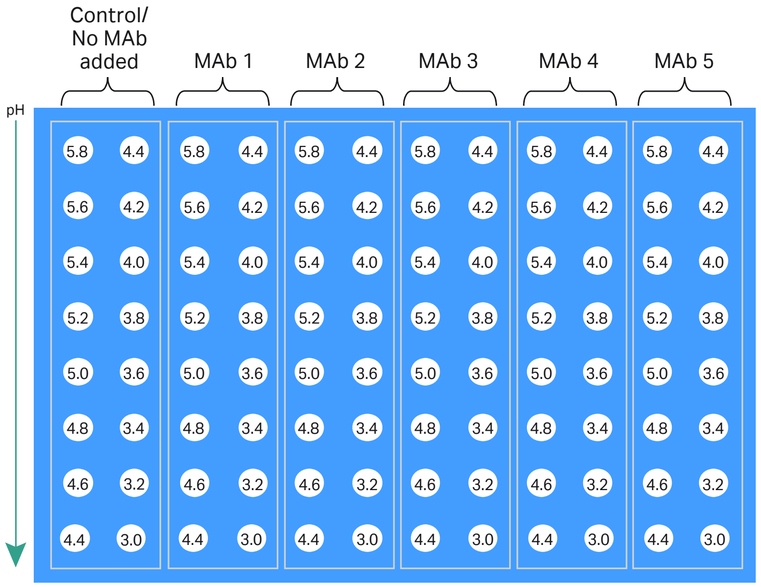


Fig 19. Plate design for the elution study using PreDictor MabSelect SuRe, 50 µL plate, a simulated pH gradient elution evaluation.

Steps:

Equilibration 1–3:	200 µL equilibration buffer, 1 min incubation
Sample loading:	300 µL MAb sample solution incubated for 20 min
Wash 1 and 2:	200 µL equilibration buffer, 1 min incubation
Wash 3:	200 µL final wash buffer, 1 min incubation
Elution 1–3:	200 µL of the different elution buffers, 1 min incubation

Liquid was removed by centrifugation and incubation was done on a shaker (1100 rpm) in all steps.

The plate layout is shown in Figure 19. All fractions were collected in UV-readable plates and the concentration of MAb in the different fractions was determined from absorbance measurements at 280 nm.

Experimental: Column chromatography

Column:	2 mL of MabSelect SuRe packed in Tricorn 5/100 column
Samples:	1.3 g/L of five different MAb's, MAb nos. 1, 3, 4, and 5 in equilibration buffer, MAb no. 2 in unpurified clarified NSO-cell supernatant were loaded to 0.7 mg MAb/mL chromatography medium
Equilibration/loading buffer:	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Wash buffer 1:	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Wash buffer 2:	20 mM sodium citrate, pH 6.0
Elution buffer:	A linear pH-gradient during 15 column volumes decreasing from pH 6 to 3

Absorbance at 280 nm was used for tracing elution.

Results

Figure 20 shows the MAb recovery in the elution fractions from the PreDictor plates together with the corresponding chromatogram from the column experiment. Good correlation between the formats was obtained. Generic elution at pH 3.6 was shown with four of the five MAbs on both formats. The fifth MAb behaved differently. In the plates it started to elute as early as pH 5.8, although most of it eluted at pH 4.8. In the column, this MAb eluted in a broad peak at pH 4.8. For further details, see Reference 12.

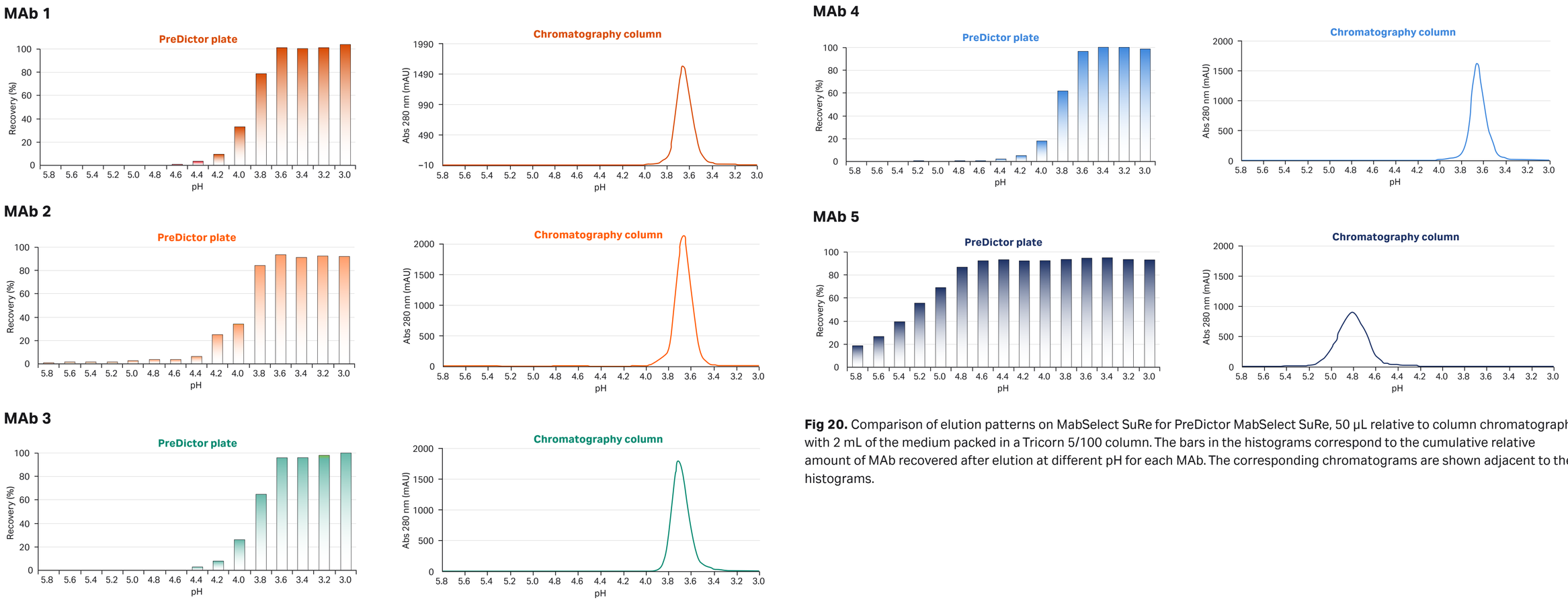


Fig 20. Comparison of elution patterns on MabSelect SuRe for PreDictor MabSelect SuRe, 50 µL relative to column chromatography with 2 mL of the medium packed in a Tricorn 5/100 column. The bars in the histograms correspond to the cumulative relative amount of MAb recovered after elution at different pH for each MAb. The corresponding chromatograms are shown adjacent to the histograms.

Comments

Note the low loads used in these experiments. In the column experiment, an analytical load (0.7 mg MAb/mL medium) was used. For simplicity, the same sample was used in the plate, which gave a load of 8 mg/mL medium. If load can affect the results, use the same load in the plates as in the column experiments. See Sections 2.6, 2.7, and 4.3 for discussion on choosing phase ratio and initial concentration in order to reach a desired load.

4.5 Rapid development of CIP protocols for affinity media

Commonly used protein-based affinity ligands such as protein A are very stable, which can allow several hundred monoclonal antibody purification cycles starting from mammalian cell culture. Critical to maximum media lifetime is the use of efficient and media-compatible cleaning-in-place (CIP) solutions. CIP protocols for MabSelect SuRe and MabSelect developed using PreDictor plates (13) are presented to the right. Note that even though CIP studies show good results, the plates should not be reused as repeated loading of protein can cause leakage.

Screening cleaning efficiency

Fouling in a protein A column can only appear after several purification cycles. Therefore, artificial fouling of the medium in 96-well filter plates was performed in order to evaluate the cleaning efficiency of various CIP conditions in a high-throughput manner (Fig 21).

Several potential CIP agents were evaluated for cleaning efficiency of the artificially fouled medium. The agents were added to the wells after step 7. Following a 15 min contact time, the cleaning solutions were removed and the wells washed several times with PBS and water. Medium samples were removed and boiled with SDS sample buffer for analysis by SDS-PAGE (Fig 22).

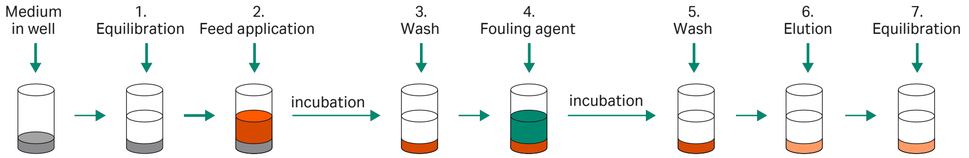
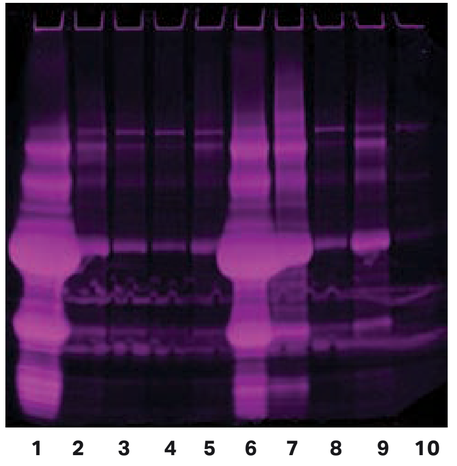


Fig 21. Schematic description of artificial fouling of media. Media in all wells were mixed at every step and liquid was removed by centrifugation between each step. Steps 1, 3, 5, and 7 show wash or equilibration with PBS, pH 7.4. Step 2 is *E. coli* lysate spiked with polyclonal human IgG (3 g/L) and added to MabSelect SuRe (PreDictor MabSelect SuRe, 50 µL medium/well). Incubation was 45 min. Step 4 shows the addition of fouling agent (2.9 M ammonium sulfate, 0.6 M phosphoric acid, pH 2.5). Incubation was overnight. Plates were covered with adhesive film to prevent evaporation. Step 6 shows sodium citrate, pH 3.0, which was applied to elute most of the bound protein.

MabSelect SuRe, agarose-based protein A medium



1. PBS (control)
2. 0.1 M NaOH
3. 0.2 M NaOH
4. 0.5 M NaOH
5. 0.1 M NaOH, 30% isopropanol
6. 0.1% commercial detergent
7. 0.1 M NaOH, 1 M NaCl
8. 0.1 M NaOH followed by 0.1 M citric acid
9. 0.1% commercial detergent followed by 0.1 M NaOH
10. Blank (control)

Fig 22. Proteins remaining on the MabSelect SuRe medium after cleaning with different agents. SDS-PAGE (Deep Purple stained) was used to evaluate cleaning efficiency. NaOH proved to be very effective.

Screening protein A media stability

Media stability was determined in a separate study by measuring functionality (i.e., IgG binding capacity) before and after exposure to cleaning chemicals.

Determination of IgG binding capacity

PreDictor 96-well filter plates: prefilled with MabSelect SuRe or MabSelect™ (6 µL medium/well) were used to determine IgG binding capacity. The binding capacity was determined by saturating the media with 200 µL of IgG at a concentration of 1.5 mg/mL. After incubating for 60 min on an orbital shaker (1100 rpm), unbound protein was collected and analyzed for IgG concentration. Binding buffer (PBS, pH 7.4) was used as wash and the bound protein was eluted with 0.1 M sodium citrate, pH 3.0. Static binding capacity was determined.

Effect of cleaning agents on chromatography media stability

PreDictor NaOH and *n*-propanol concentrations were varied between 0.01 and 1.09 M and 0% to 10%, respectively. The media were stored in the CIP solution for 18 h, which corresponds to 180 cycles with 30 min CIP/Sanitization-in-place (SIP) every fifth cycle. MODDE™ software (Umetrics, reference 14) was used for setting up the experiments and evaluating the results (Fig 23).

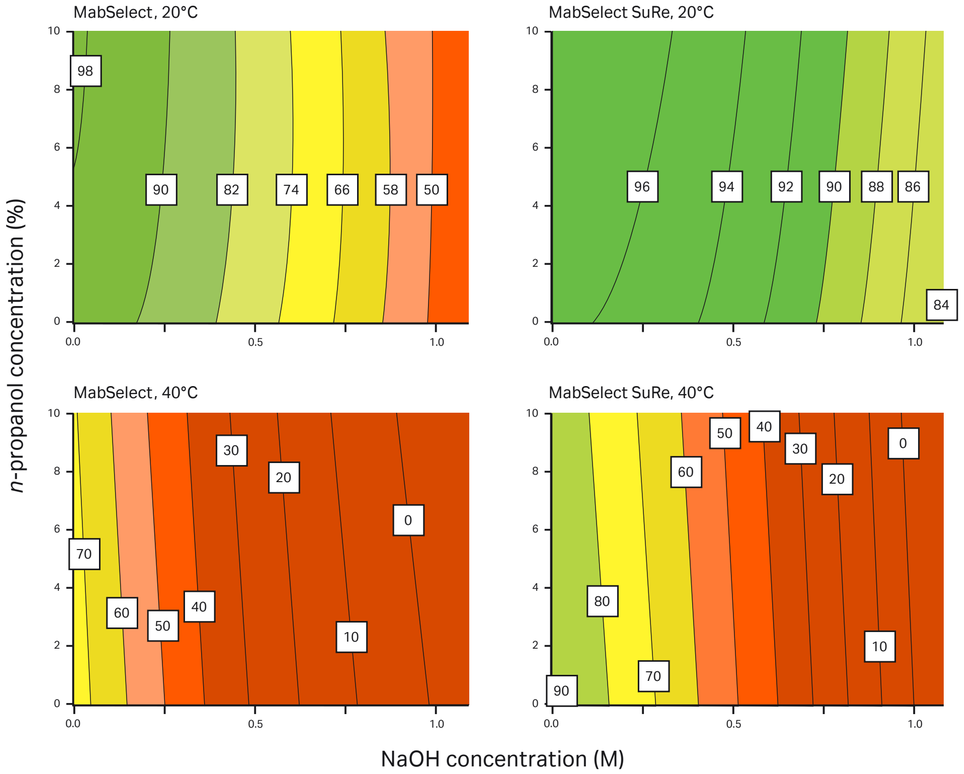


Fig 23. Contour plots showing remaining IgG binding capacity (%) after exposure to CIP chemicals. PreDictor MabSelect SuRe (alkali-stabilized protein A maintained IgG binding capacity at higher NaOH concentrations and elevated temperatures compared with PreDictor MabSelect (recombinant protein A).

Conclusions

PreDictor 96-well filter plates filled with chromatography media enable parallel screening of CIP agents for cleaning efficiency and media compatibility studies. This approach allows a rapid design of suitable CIP protocols for different media and feed-stock combinations.

NaOH at concentrations from 0.1 to 0.5 M proved effective for cleaning agarose-based protein A media fouled with *E. coli* lysate.

4.6 Adsorption isotherms with PreDictor plates

Adsorption isotherms were constructed for adsorption of human IgG on MabSelect SuRe. A PreDictor MabSelect SuRe adsorption isotherm plate was used and isotherms were constructed from flowthrough and elution data.

Experimental:

Plate:	PreDictor MabSelect SuRe isotherm
Equilibration/loading buffer:	20 mM sodium phosphate, pH 7.4
Wash buffer:	20 mM sodium phosphate, pH 7.4
Elution buffer:	20 mM sodium citrate, pH 3.6, 150 mM NaCl
Sample:	Polyclonal human IgG, hIgG (Gammanorm™, Octapharma AB) at concentration 4.0 g/L in equilibration/loading buffer

Steps:

Equilibration 1–3:	200 µL equilibration buffer, 1 min incubation
Sample loading:	200 µL sample incubated for 6 h
Wash 1–3:	200 µL equilibration buffer, 1 min incubation
Elution 1–3:	200 µL elution buffer, 1 min incubation

Mixing was done on an orbital shaker at 1100 rpm in all steps. Liquids were removed by vacuum filtration.

Calculations:

UV absorbance was used to calculate protein concentration. Binding capacities, expressed as mass of protein per unit sedimented medium, were calculated from flowthrough data or elution data.

For flowthrough data, c was measured directly from the flowthrough and binding capacity, q calculated from the mass balance equation (Eq. 6). Retained liquid, V_{liq} (see Section 2.3) was accounted for in the calculations.

Elution was performed in three consecutive elutions. Binding capacity, q, was calculated according to Equation 16 summarizing the amount of protein found in each elution (i). The concentration of the target protein, c, was calculated according to Equation 17.

$$q = \frac{\sum_{1}^{i} V_{eluate,\,i} \times c_{eluate,\,i}}{V_{medium}} \qquad (16)$$

$$c = c_o - q\,\frac{V_{medium}}{V_{liq}} \qquad (17)$$

	1	2	3	4	5	6	7	8	9	10	11	12
A	50 µL	50 µL	20 µL	20 µL	8 µL	8 µL	6 µL	6 µL	4 µL	4 µL	2 µL	2 µL
B	50 µL	50 µL	20 µL	20 µL	8 µL	8 µL	6 µL	6 µL	4 µL	4 µL	2 µL	2 µL
C	50 µL	50 µL	20 µL	20 µL	8 µL	8 µL	6 µL	6 µL	4 µL	4 µL	2 µL	2 µL
D	50 µL	50 µL	20 µL	20 µL	8 µL	8 µL	6 µL	6 µL	4 µL	4 µL	2 µL	2 µL
E	50 µL	50 µL	20 µL	20 µL	8 µL	8 µL	6 µL	6 µL	4 µL	4 µL	2 µL	2 µL
F	50 µL	50 µL	20 µL	20 µL	8 µL	8 µL	6 µL	6 µL	4 µL	4 µL	2 µL	2 µL
G	50 µL	50 µL	20 µL	20 µL	8 µL	8 µL	6 µL	6 µL	4 µL	4 µL	2 µL	2 µL
H	50 µL	50 µL	20 µL	20 µL	8 µL	8 µL	6 µL	6 µL	4 µL	4 µL	2 µL	2 µL

Fig 24. Plate layout for the adsorption isotherm experiment using PreDictor MabSelect SuRe isotherm plate. Values indicate volumes of MabSelect SuRe in the wells.

Results and discussion

The obtained isotherms are shown in Fig 25. Values of the parameters in the Langmuir adsorption isotherm (Eq. 13) were estimated from capacities calculated from flowthrough and elution data. The least square estimates for flowthrough data were q_{max} = 57 g/L and equilibrium dissociation constant, K_d = 0.15 g/L, respectively. For elution data, the least square estimates were q_{max} = 59 g/L and K_d = 0.25 g/L, respectively.

As mentioned in Section 2.9.6, several different approaches can be used to obtain an isotherm. If single-medium plates are used, the concentration of sample added to the wells can be varied. Isotherm plates containing variable volumes of medium in the wells are an alternative. In this experiment, the latter approach was chosen to avoid sample dilution and volume adjustment.

To illustrate the results, capacities were calculated either from flowthrough data or from elution data. In this application, similar results were obtained with both approaches. Note that if elution data are used to create adsorption isotherms, all of the bound material is assumed to be collected in the elution fractions i.e., elution conditions are optimal. Alternatively, flowthrough data may be used to construct the adsorption isotherm.

Use flowthrough data when constructing the isotherm.

For further reading on adsorption isotherms, see Reference 15.

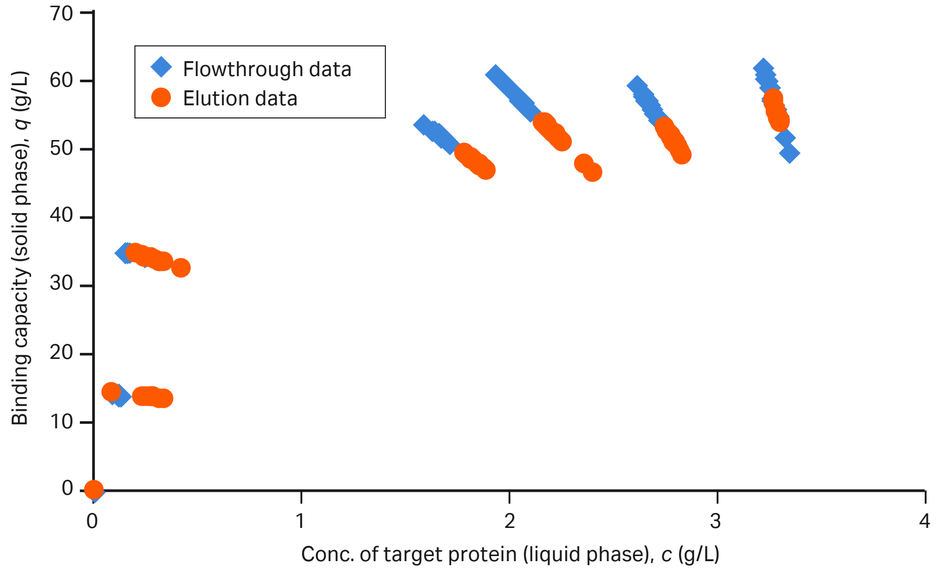


Fig 25. Adsorption isotherms for hIgG on MabSelect SuRe. Capacities were calculated either from flowthrough data (blue) or elution data (orange).

05

Quality aspects of the PreDictor plate workflow

An important aspect of using PreDictor plates is that chromatography media volume reproducibility is good both between wells within a plate and between plates. Studies of reproducibility between wells and plates, replication, and method variability vs media volume variability are presented below (16).

5.1 Functional testing of reproducibility

The reproducibility of PreDictor plates was studied by testing the binding capacity of 113 plates from continuous production. Seven media were tested and the volume in each well was measured. Each plate thus gave 96 data points and 10 848 wells were tested in total. High reproducibility within plates was obtained in all cases. Relative standard deviations (RSDs) were in the order of 1% to 5% (Fig 26).

5.2 Increasing the robustness of experiments

Even though throughput can be maximized by performing singular measurements, replication of experiments is generally recommended. This increases the robustness of the batch experiment by reducing variability and decreasing the risk of repeated runs for specific conditions. The estimated variability within and between plates was used to calculate 95% confidence intervals for triplicate well measurements (Fig 27).

The uncertainty (+/-) in binding capacity of triplicate samples was in all cases less than 7%. This uncertainty decreases at larger media volumes. However, measuring binding capacity with larger volumes can require unrealistically high initial protein concentrations or excessive sample volumes.

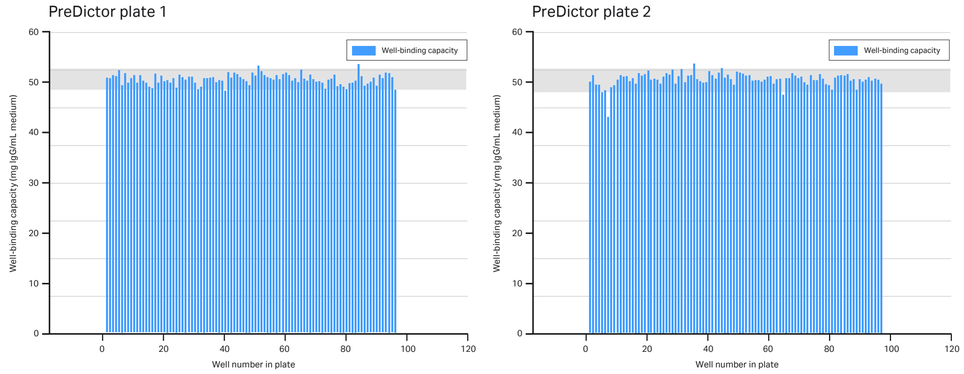


Fig 26. Evaluation of the hIgG-binding capacity of two different PreDictor MabSelect SuRe, 6 µL plates. The gray horizontal bands denote 95% confidence limits for each plate. Each veritical blue bar denotes the binding capacity for a specific well. The Relative Standard Deviation (RSD) was 1% to 1.2% in each plate.

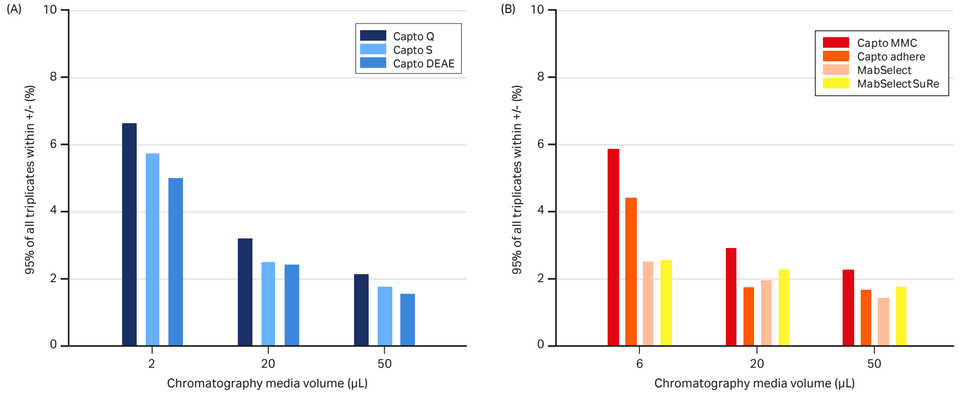


Fig 27. (A) Reproducibility of PreDictor plates filled with ion exchangers Capto Q, Capto S, and Capto DEAE tested with different proteins. (B) Reproducibility of PreDictor Capto MMC, Capto adhere, MabSelect, and MabSelect SuRe tested with polyclonal IgG and BSA.

5.3 Method variability vs chromatography media volume variability

Blank run variability was used as an estimate of “non-media volume-related” error sources (e.g., factors such as pipetting, plate handling, and detection). UV detection was chosen for this study as it is well-suited for the amounts of protein used. In this case, the contribution from media volume variability was similar to the contribution from plate handling and detection (Fig 28).

The relative influence of chromatography media volume variability on the total end uncertainty of an experiment may be even smaller when less precise detection methods are used (Fig 29).

Assay replication should be considered if less precise detection methods are used. However, as stated earlier, true experimental replication is still recommended to ensure robust and efficient process development in PreDictor plates.

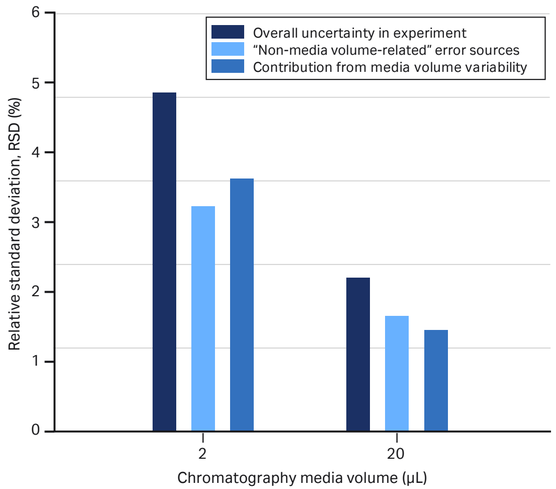


Fig 28. Overall RSD and variability contributions from method handling and chromatography media volume.

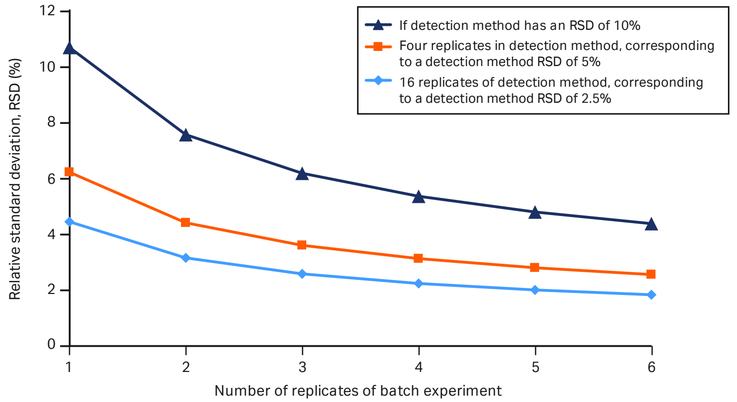


Fig 29. The effect of replicating a detection method with a relative standard deviation (RSD) of 10% on the overall uncertainty in the experiment. Different curves show the effect of changing the number of detection replicates. The x-axis shows the effect of replication of the batch experiment. A detection RSD of 2.5% corresponds roughly to UV measurements in PreDictor plates. Calculations are based on a 2 µL chromatography medium volume.

Conclusions

The reproducibility of chromatography media volume in PreDictor plates is good (RSD in binding capacity of 1.5% to 5% between wells). Replicate experiments should be performed to maintain the robustness of the high-throughput approach in PreDictor plates. The uncertainty in detection methods is of more concern than the chromatography media volume variability, which is the same level as error sources from UV-detection and liquid handling.

06

Assist software

6.1 Assist software workflow

Assist software helps chromatography process developers design and evaluate PreDictor plate experiments by facilitating the high-throughput methodology. The software supports all steps in the workflow by:

- Providing guidance to design experiments
- Handling experimental data relating to an experiment
- Providing tools for data analysis

Figure 30 shows the Assist software workflow.

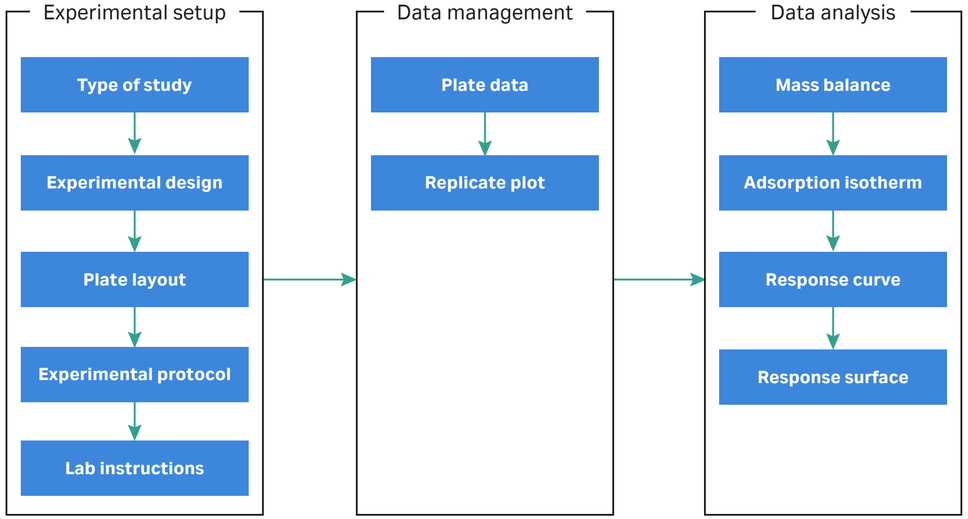


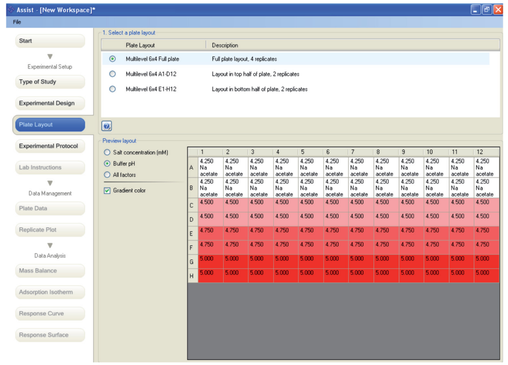
Fig 30. Assist software workflow.

6.1.1 Experimental setup

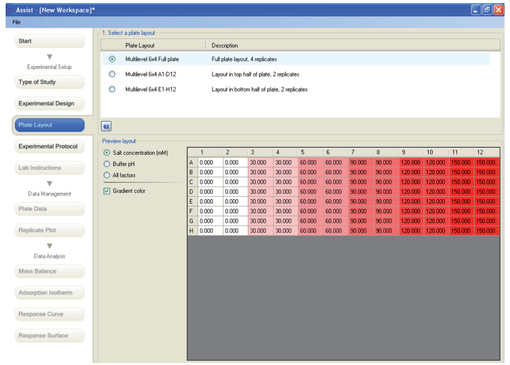
Based on the type of study (i.e., binding, wash, elution, determining adsorption isotherms, or media screening), and on the number of experimental factors (e.g., salt concentration, pH, buffer system, etc.), Assist software suggests different experimental designs that map out the experimental factors in a 96-well filter plate. These designs are suitable for automated workflows using robotic systems or manual workflows using multi-channel pipettes. Importing custom designs into the software using a supplied Microsoft™ Excel™ file is also possible. Since the 96-well plate format allows testing many different experimental conditions simultaneously, the designs include many different factor levels. The physical layout of factors is shown in the plate layout section, either one-by-one as colored gradients in the plate, or all at the same time (see Fig 31).

A detailed laboratory protocol based on the experimental steps in the workflow is created in the experimental protocol section. This can be modified and other documentation such as notes, can be added for traceability. The protocol can be printed and used as a laboratory instruction when setting up the experiment, and saved for future documentation.

Buffer pH



Salt concentration



All factors

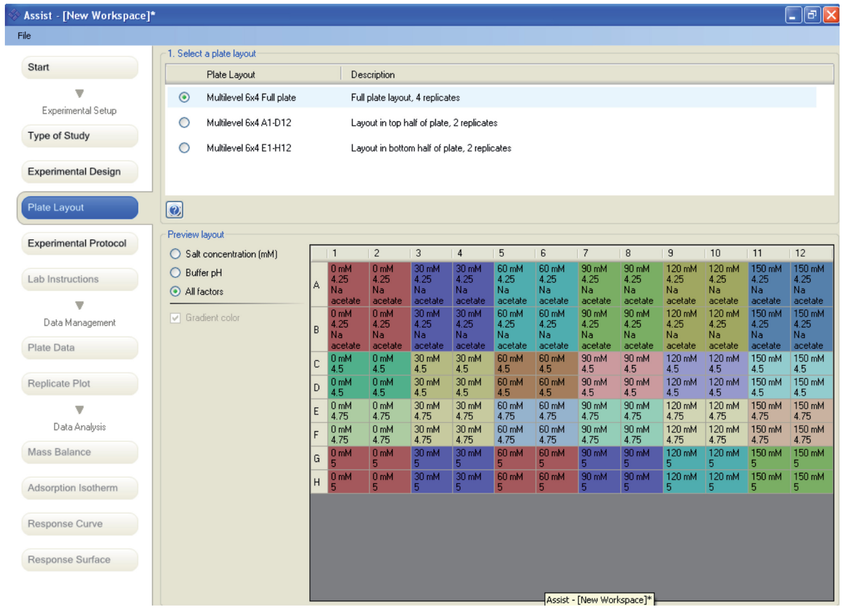


Fig 31. Factor level layout is either shown one-by-one as colored gradients (e.g., buffer pH) or all at the same time.

6.1.2 Data management

The data management section includes two parts: Data loading and raw data management.

Data can be loaded from a file or pasted from the clipboard, either as concentration (µg/µL), mass (µg), UV absorbance (AU) or a generic response (any kind of data, e.g., impurity data).

Raw data management displays data both in table format and as a replicate plot. The table presents calculated mean values, standard deviations and relative standard deviations (RSD) for the replicates. A statistical way to identify and/or exclude outliers is not available, but a sorting function makes it easy to identify replicates with large variation. Data points can easily be excluded from further analysis and re-included into the analysis again. Excluded data points are indicated in the replicate plot, but not displayed in subsequent plots.

6.1.3 Data analysis

Data evaluation includes calculating and visualizing mass balance, response curves, and response plots. In this way, the effects of experimental conditions on responses such as binding capacity or yield can easily be identified.

The mass balance section shows the mass that has been recovered in each well and for each experimental step where analysis has been performed and data loaded into Assist (see above). This functions as a quality check for the overall recovery and allows detection of outliers.

The Response curve (Fig 32) shows a response as a function of one experimental factor, while the Response surface (Fig 33) shows the response as a function of two factors. The first is displayed as a curve and the second as a colored surface based on interpolated data showing the actual experimental results as crosses.

Plots can be exported in enhanced metafile format and the tables can be exported as tab-separated text files. They can also be used in other applications such as statistical modeling of the Response surface.

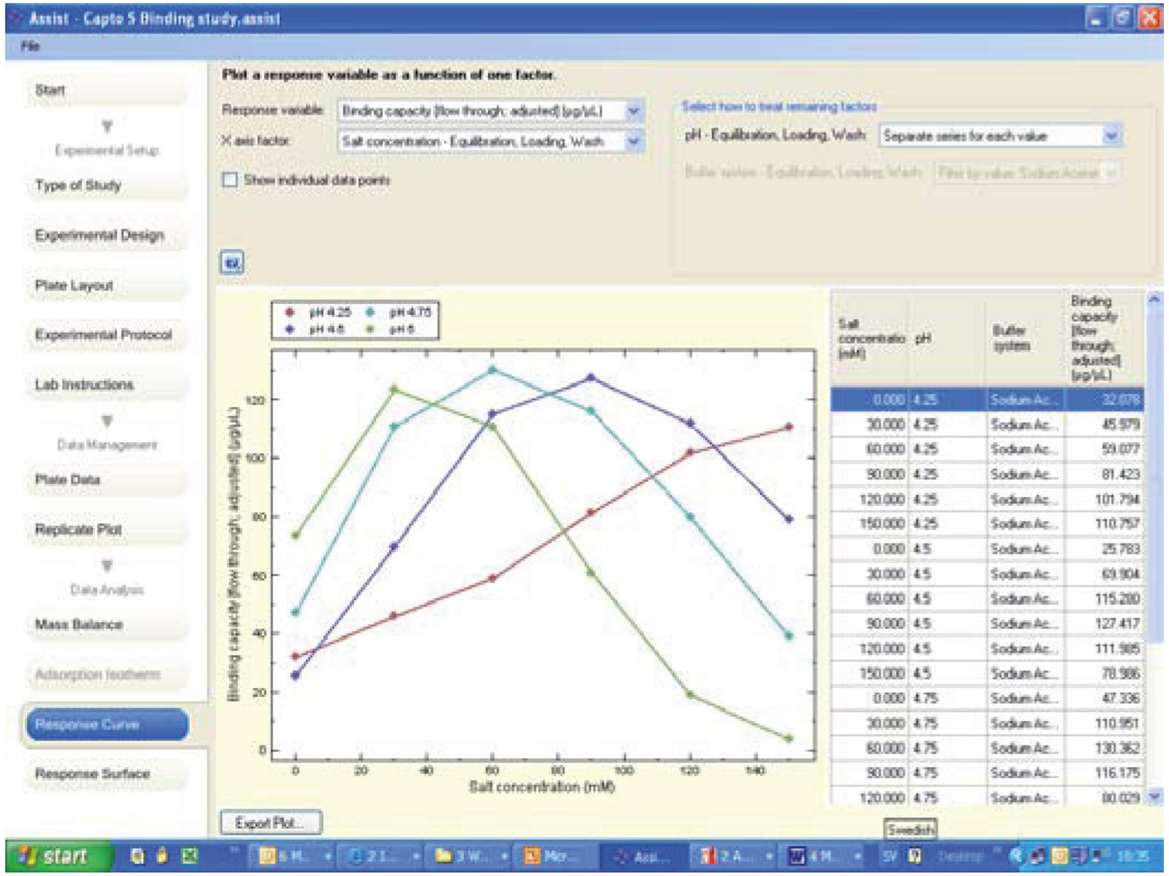


Fig 32. Screen dump showing a Response curve, which shows a response as a function of a single experimental factor.

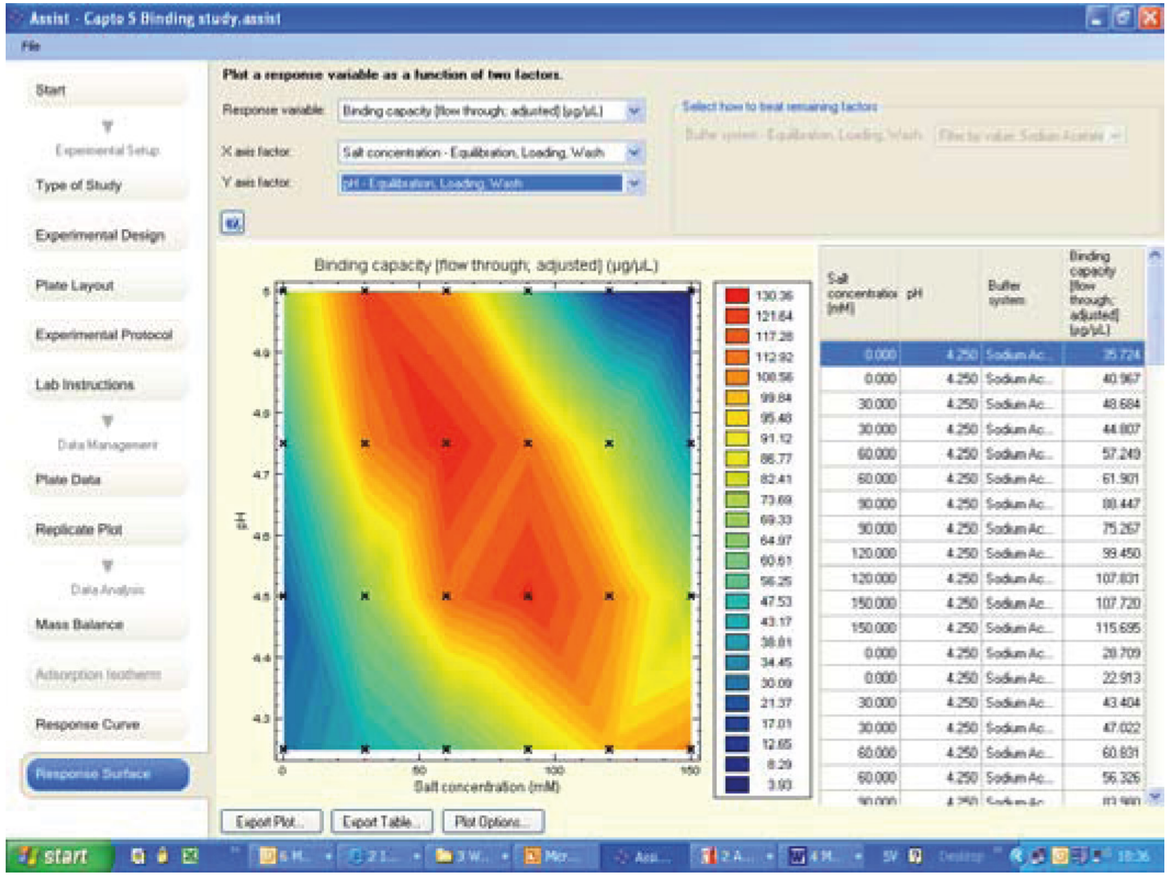


Fig 33. Screen dump showing a Response surface, which shows a response as a function of two experimental factors. The colored surface is based on interpolated data showing the actual experimental results as crosses.

Adsorption isotherm panes are only available when setting up an adsorption isotherm study. In adsorption isotherm analysis, a Langmuir isotherm is fitted to the data points to determine the equilibrium dissociation constant K_d (µg/µL) and the maximum capacity q_{max} (µg/µL) from either flowthrough data or from elution data.

07

PreDictor plate selection

7.1 PreDictor Plate selection guide

1. Single-medium plates

- For binding, wash, or elution studies
- Same medium volume throughout the plate
- Plates with three different volumes available. Type of study governs which volume to use (refer to Table 2).

2. Media-screening plates

- For binding, wash, and elution studies on multiple chromatography media
- Two types of plates available: 1) Anion- and multimodal anion-exchange media plate (Capto Q, Capto DEAE, Q Sepharose Fast Flow, and Capto adhere), 2) cation and multimodal cation exchange media plate (Capto S, SP Sepharose Fast Flow, and Capto MMC). For plate layout, see Section 7.2.
- Plates with two different media volumes are available. Type of study governs which volume to use (refer to Table 2).

3. Adsorption isotherm plates

- Used for binding studies done under equilibrium conditions to obtain fundamental thermodynamic understanding of the adsorption process
- Single-medium per plate, but with varied medium volume in the wells. This facilitates simple and rapid construction of the isotherm as sample manipulation is minimized by avoiding the need to vary the concentration of sample molecule between wells. For plate layout, see Section 7.2.

4. Custom plates

- Please contact your Cytiva representative to discuss the availability of customized PreDictor plates for your process development needs

When choosing PreDictor plates, consider the amount of material, target protein, and impurities required for final analysis. If a large amount of sample is needed, a larger medium volume and/or increased number of sample aliquots in the loading step is necessary. Alternatively, several replicates from one plate can be pooled.

For binding studies, relatively small volumes of chromatography medium are used. The medium should be overloaded with protein and the amount of unbound protein measured. Alternatively, the amount of bound protein is determined from the elution pool. Generally, single-medium plates with 2 or 6 µL volumes should be used, but in some cases plates with 20 µL or 50 µL can be employed. Check the suitability of a particular plate by performing a rough calculation as described in the example in Section 2.9.1. The properties of the medium dictate the volumes used; for high-capacity ion exchangers, 2 µL is sufficient, while for the other media, 6 µL is required for optimal results. For wash and elution studies, larger medium volumes can be required if sample purity needs to be determined. In such cases, the minimum detectable amount of impurities will govern the choice of PreDictor plate. The first option for wash and elution studies is the 20 µL plate.

The screening plates facilitate media screening. Instead of using several single-medium plates to screen different media, one plate contains three (PreDictor CIEX screening plate) or four (PreDictor AIEX screening plate) different media.

Adsorption isotherm plates contain wells with media volumes ranging from 2 to 50 µL, which ensures that the amount of target protein bound and final concentration of target protein in the supernatant varies so that an isotherm (capacity vs equilibrium concentration) can be constructed.

Table 2. Predictor plate selection guide by application

Single-medium plates — Application: Binding conditions

Medium volume in well (µL)[1]	Capto Q	Capto S	Capto DEAE	Capto MMC	Capto adhere	Q Sepharose Fast Flow	SP Sepharose Fast Flow	MabSelect family
2	++	++	++	NA	NA	NA	NA	NA
6	NA	NA	NA	++	++	++	++	++
20	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—

Single-medium plates — Application: Wash/elution conditions

Medium volume in well (µL)[1]	Capto Q	Capto S	Capto DEAE	Capto MMC	Capto adhere	Q Sepharose Fast Flow	SP Sepharose Fast Flow	MabSelect family
2	—	—	—	NA	NA	NA	NA	NA
6	NA	NA	NA	—	—	—	—	—
20	++[2]	++[2]	++[2]	++[2]	++[2]	++[2]	++[2]	++[2]
50	+[3]	+[3]	+[3]	+[3]	+[3]	+[3]	+[3]	+[3]

Media-screening plates — Application: Binding conditions

Medium volume in well (µL)[1]	AIEX screening plate[4]	CIEX screening plate[4]
2 and 6	++	++
20	—	—

Media-screening plates — Application: Wash/elution conditions

Medium volume in well (µL)[1]	AIEX screening plate[4]	CIEX screening plate[4]
2 and 6	—	—
20	++	++

Adsorption isotherm plates — Application: Adsorption isotherm studies

Medium volume in well (µL)[1]	Variable (2, 4, 6, 8, 20, and 50 µL). Only one type of plate per medium available

++ First choice
\+ Possible
— Not recommended
NA Product not available
[1] Corresponds to sedimented volume
[2] The 20 µL plate is the preferred plate for the first set of experiments.
[3] The 50 µL plate is used for when protein concentration is high or when large amounts of sample are needed for analysis.
[4] AIEX screening plate contains Capto Q, Capto DEAE, Q Sepharose Fast Flow, and the multimodal anion exchanger Capto adhere. CIEX screening plate contains Capto S, SP Sepharose Fast Flow, and the multimodal cation exchanger Capto MMC.

7.2 Available plates

Single-medium plate

Medium volume in well (µL)	Row		Column 1	2	3	4	5	6	7	8	9	10	11	12
2*	A–H	V_{medium}	2	2	2	2	2	2	2	2	2	2	2	2
		$V_{storage}$†	200	200	200	200	200	200	200	200	200	200	200	200
6*	A–H	V_{medium}	6	6	6	6	6	6	6	6	6	6	6	6
		$V_{storage}$†	500	500	500	500	500	500	500	500	500	500	500	500
20*	A–H	V_{medium}	20	20	20	20	20	20	20	20	20	20	20	20
		$V_{storage}$†	500	500	500	500	500	500	500	500	500	500	500	500
50*	A–H	V_{medium}	50	50	50	50	50	50	50	50	50	50	50	50
		$V_{storage}$†	500	500	500	500	500	500	500	500	500	500	500	500

* Capto S, Capto DEAE, Capto S available as 2, 20, 50 µL plates. MabSelect, MabSelect SuRe, MabSelect Xtra™, Q Sepharose Fast Flow, SP Sepharose Fast Flow, Capto MMC, and Capto adhere available as 6, 20, or 50 µL plates.

† $V_{storage}$: Volume of storage solution in the well. Storage solution is 20% ethanol for all media except SP Sepharose Fast Flow and Capto S, where the storage solution is 20% ethanol with 0.2 M sodium acetate.

AIEX screening plate

Medium volume in well (µL)	Row		Column 1	2	3	4	5	6	7	8	9	10	11	12
			Capto Q			Capto DEAE			Q Sepharose Fast Flow			Capto adhere		
2 or 6	A–H	V_{medium}	2	2	2	2	2	2	6	6	6	6	6	6
		$V_{storage}$	200	200	200	200	200	200	500	500	500	500	500	500
20	A–H	V_{medium}	20	20	20	20	20	20	20	20	20	20	20	20
		$V_{storage}$	500	500	500	500	500	500	500	500	500	500	500	500

7.2 Available plates

CIEX screening plate

Medium volume in well (µL)			Column											
			Capto S				SP Sepharose Fast Flow				Capto MMC			
	Row		1	2	3	4	5	6	7	8	9	10	11	12
2 or 6	A–H	V_{medium}	2	2	2	2	6	6	6	6	6	6	6	6
		$V_{storage}$	200	200	200	200	500	500	500	500	500	500	500	500
20	A–H	V_{medium}	20	20	20	20	20	20	20	20	20	20	20	20
		$V_{storage}$	500	500	500	500	500	500	500	500	500	500	500	500

Adsorption isotherm plate*

Medium volume in well (µL)			Column											
	Row		1	2	3	4	5	6	7	8	9	10	11	12
2 to 50	A–H	V_{medium}	50	50	20	20	8	8	6	6	4	4	2	2
		$V_{storage}$	500	500	200	200	500	500	375	375	250	250	125	125

* Same plate design for all media

08

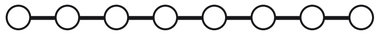
Experimental designs/setups

The 96-well plate format allows a large number of experiments to be performed in parallel. Experimental design can vary from single-factor designs to more advanced DoE designs (Fig 34). DoE designs are used to acquire as much information as possible in as few experiments as possible. Because of the large number of experiments that can be performed, information from experimental data can yield information similar to that obtained from statistical modeling of responses. This is shown in Figure 16, which shows surface plots derived from raw data. In general, a larger experimental space can be covered by the plate format because of scale-down effects and parallellization.

(A)

Factor 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00
B	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71
C	-0.43	-0.43	-0.43	-0.43	-0.43	-0.43	-0.43	-0.43	-0.43	-0.43	-0.43	-0.43
D	-0.14	-0.14	-0.14	-0.14	-0.14	-0.14	-0.14	-0.14	-0.14	-0.14	-0.14	-0.14
E	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
F	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43
G	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00



(B)

Factor 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
B	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
C	1	0	1	0	1	0	1	0	1	0	1	0
D	1	0	1	0	1	0	1	0	1	0	1	0
E	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
F	-1	1	-1	1	-1	1	-1	1	-1	1	-1	1
G	1	0	1	0	1	0	1	0	1	0	1	0
H	1	0	1	0	1	0	1	0	1	0	1	0

Factor 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
B	1	-1	1	-1	1	-1	1	-1	1	-1	1	-1
C	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
D	1	1	1	1	1	1	1	1	1	1	1	1
E	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
F	1	0	1	0	1	0	1	0	1	0	1	0
G	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
H	1	0	1	0	1	0	1	0	1	0	1	0

Factor 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
B	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
C	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
D	-1	0	-1	0	-1	0	-1	0	-1	0	-1	0
E	1	0	1	0	1	0	1	0	1	0	1	0
F	1	0	1	0	1	0	1	0	1	0	1	0
G	1	0	1	0	1	0	1	0	1	0	1	0
H	1	0	1	0	1	0	1	0	1	0	1	0

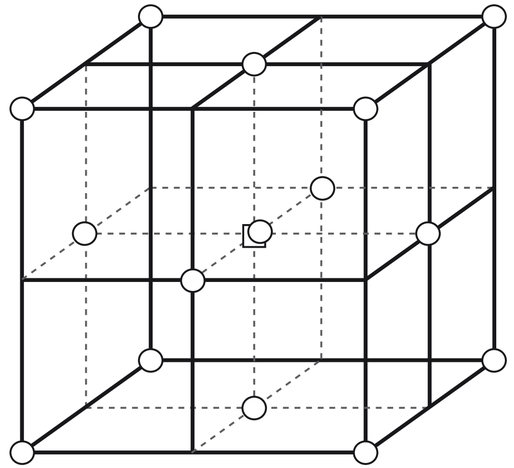


Fig 34. Examples of experimental designs/setups. Factor level range from -1 to 1. (A) One factor in 8 levels with 12 replicates. (B) Central Composite Face design (CCF); eight corner points, six face points, and two center-points. Mapped out in a full PreDictor plate, the design results in six replicates.

09

Steps in process development following screening

9.1 HiScreen and HiTrap columns

Prepacked HiScreen columns (Fig 35) are the natural next step from PreDictor plates for optimization and verification, or further process development studies on binding capacity, robustness, and resolution. Nineteen HiScreen columns are currently available, packed with different media from the MabSelect, Capto, and Sepharose families for affinity chromatography, ion exchange chromatography, and hydrophobic interaction chromatography. HiScreen columns are prepacked with the same chromatography media that are prefilled in PreDictor plates, which ensures reproducible results when scaling up. HiScreen columns have 10 cm bed height and a small volume (4.7 mL) to keep sample and buffer consumption low. If a larger bed height is required, two columns can be connected in series to achieve a 20 cm bed.

In antibody purification, binding capacity is one of the most important parameters. HiScreen columns are a useful tool for verification studies. In Figure 36, dynamic binding capacity was measured at three different residence times for HiScreen MabSelect, HiScreen MabSelect SuRe, and HiScreen MabSelect Xtra. For this monoclonal antibody, HiScreen MabSelect Xtra showed the highest capacity.

For more information visit www.cytiva.com/protein-purification

HiTrap columns in convenient 1 mL and 5 mL sizes complement PreDictor plates and offer an alternative to HiScreen columns if sample volumes are limited. HiTrap columns are available prepacked with the same chromatography media that are available in PreDictor plates and HiScreen columns.

For more information, visit www.cytiva.com/hitrap

Fig 35. HiScreen columns are available prepacked with a wide range of chromatography media for screening and optimization studies.

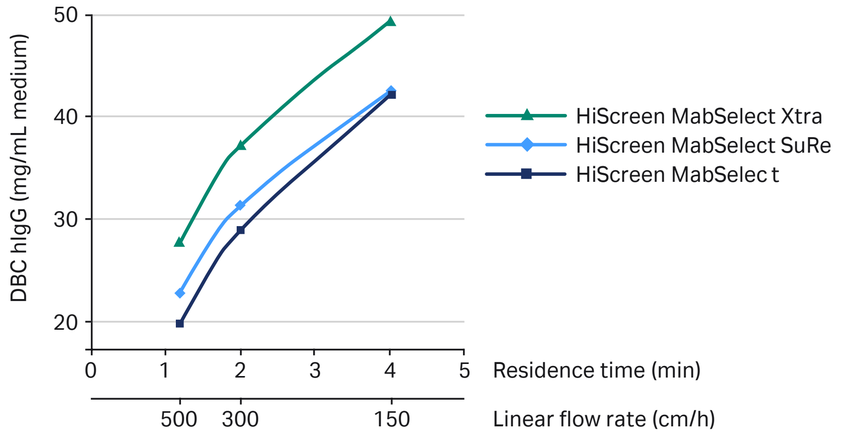


Fig 36. Comparison of dynamic binding capacity for hIgG on the three columns available for antibody purification.

9.2 ÄKTA chromatography systems and UNICORN control software

Prepacked HiScreen and HiTrap columns are used with ÄKTA design systems such as ÄKTA avant 25 chromatography system, a liquid chromatography system for chromatography media screening, method scouting, and fast, automated process development.

Increased productivity, more efficient method scouting and process development is a key feature of ÄKTA avant 25, which has flow rates and pressure specifications that support use of Cytiva's BioProcess™ media such as MabSelect and Capto. The system, which comprises advanced instrumentation and specially developed UNICORN 6 control software also offers greater security through column recognition and individual column run history data, automatic on-line buffer preparation, and easy protocol transfer during scale-up. An important feature of the UNICORN 6 control software is the Design of Experiments tool, which enables increased efficiency in process development and provides time and cost savings by capturing more precise information in fewer experiments.

For more information, visit www.cytiva.com/akta

Fig 37. ÄKTA avant 25 is a high-performance system designed for process development, method optimization, and scouting.

9.3 BioProcess media

Cytiva's BioProcess media cover all purification steps from capture to polishing, for all scales of work from development and routine production. BioProcess media are developed for production-scale chromatography to meet the demands of industrial biotechnology. All media are manufactured with validated methods and tested to meet stringent quality requirements. Regulatory Support Files are available to assist process validation and submissions to regulatory authorities.

Columns and systems for purification scale-up are also available from Cytiva, see www.cytiva.com/bioprocess for more information

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11
Nomenclature

Common acronyms and abbreviations

m_{added}	Mass of target protein added to the well
m_{bound}	Mass of target protein bound to the chromatography medium
$m_{unbound}$	Mass of target protein not bound to the chromatography medium
m_{eluate}	Mass of target protein in eluate fraction(s)
m_{FT}	Mass of target protein in flowthrough fraction
m_{wash}	Mass of target protein in the wash fraction(s)
c	Concentration of target protein in the liquid phase
c_{eq}	Concentration of target protein in the liquid phase at equilibrium
c_{o}	Initial concentration of target protein in the liquid phase at the start of the experiment, see Section 2.3
c_{sample}	Concentration of target protein in the sample
c_{eluate}	Concentration of target protein in eluate fraction
q	Concentration of target protein in the solid phase (chromatography medium)
q_{max}	Maximum capacity of the chromatography medium for the target protein (saturation capacity)
V_{sample}	Sample volume
V_{liq}	Volume of the liquid phase, see Section 2.3
V_{medium}	Chromatography medium volume
V_{eluate}	Volume of eluate fraction
V_{r}	Volume of retained liquid in a well, see Section 2.3
β	Phase ratio = V_{liq}/V_{medium} , see Section 2.6
K_{d}	Equilibrium dissociation constant

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Ordering information

PreDictor plates

Single-medium plates	Quantity	Code no.
PreDictor Capto Q, 2 µL	4 × 96-well filter plates	28925773
PreDictor Capto Q, 20 µL		28925806
PreDictor Capto Q, 50 µL		28925807
PreDictor Capto S, 2 µL		28925808
PreDictor Capto S, 20 µL		28925809
PreDictor Capto S, 50 µL		28925810
PreDictor Capto DEAE, 2 µL		28925811
PreDictor Capto DEAE, 20 µL		28925812
PreDictor Capto DEAE, 50 µL		28925813
PreDictor Capto MMC, 6 µL		28925814
PreDictor Capto MMC, 20 µL		28925815
PreDictor Capto MMC, 50 µL		28925816
PreDictor Capto adhere, 6 µL		28925817
PreDictor Capto adhere, 20 µL		28925818
PreDictor Capto adhere, 50 µL		28925819
PreDictor MabSelect, 6 µL		28925820
PreDictor MabSelect, 20 µL		28925821
PreDictor MabSelect, 50 µL		28925822
PreDictor MabSelect SuRe, 6 µL		28925823
PreDictor MabSelect SuRe, 20 µL		28925824
PreDictor MabSelect SuRe, 50 µL		28925825
PreDictor MabSelect Xtra, 6 µL		28943275
PreDictor MabSelect Xtra, 20 µL		28943276
PreDictor MabSelect Xtra, 50 µL		28943277

Single-medium plates	Quantity	Code no.
PreDictor Q Sepharose Fast Flow, 6 µL	4 × 96-well filter plates	28943269
PreDictor Q Sepharose Fast Flow, 20 µL		28943270
PreDictor Q Sepharose Fast Flow, 50 µL		28943271
PreDictor SP Sepharose Fast Flow, 6 µL		28943272
PreDictor SP Sepharose Fast Flow, 20 µL		28943273
PreDictor SP Sepharose Fast Flow, 50 µL		28943274

Screening plates	Quantity	Code no.
PreDictor AIEX screening 2 µL/6 µL	4 × 96-well filter plates	28943288
PreDictor AIEX screening 20 µL		28943289
PreDictor CIEX screening 2 µL/6 µL		28943290
PreDictor CIEX screening 20 µL		28943291

Adsorption isotherm plates	Quantity	Code no.
PreDictor Capto Q isotherm[1]	4 × 96-well filter plates	28943278
PreDictor Capto S isotherm[1]		28943279
PreDictor Capto DEAE isotherm[1]		28943280
PreDictor Capto MMC isotherm[1]		28943281
PreDictor Capto adhere isotherm[1]		28943282
PreDictor MabSelect isotherm[1]		28943283
PreDictor MabSelect SuRe isotherm[1]		28943284
PreDictor MabSelect Xtra isotherm[1]		28943285
PreDictor Q Sepharose Fast Flow isotherm[1]		28943286
PreDictor SP Sepharose Fast Flow isotherm[1]		28943287

[1] Plates are manufactured on request.

Software

	Code no.
Assist 1.1 Software package	28945396
Assist 1-user license 1.1	28945397

Related products

Accessories	Quantity	Code no.
Collection plate 96-well 500 µL, V-shaped bottom (not UV-readable)	5 × 96 well-plates	28403943
Microplate foil (96-well)	100 × self-adhesive, transparent plastic foils	BR100578

Prepacked columns*	Quantity	Code no.
HiScreen Capto Q	1 × 4.7 mL	28926978
HiScreen Capto S		28926979
HiScreen Capto DEAE		28926982
HiScreen Capto MMC		28926980
HiScreen Capto adhere		28926981
HiScreen MabSelect		28926973
HiScreen MabSelect SuRe		28926977
HiScreen MabSelect Xtra		28926976
HiScreen IMAC FF		28950517
HiScreen Q FF		28950510
HiScreen SP FF		28950513
HiScreen Q HP		28950511
HiScreen SP HP		28950515
HiScreen Phenyl HP		28950516
HiScreen Butyl FF		28926984
HiScreen Butyl-S FF		28926985
HiScreen Octyl FF		28926986
HiScreen Phenyl FF (high sub)		28926988
HiScreen Phenyl FF (low sub)		28926989

* HiTrap columns prepacked with the equivalent chromatography media found in HiScreen columns are available in 5 × 1 mL and 5 × 5 mL pack sizes. Visit www.cytiva.com/hitrap for information.

Related literature

PreDictor literature	Code no.
Data file: PreDictor 96-well filter plates and Assist software	28925839
Application note: Screening of loading conditions on Capto S using a new high-throughput format, PreDictor plates	28925840
Poster: High-throughput screening of elution conditions on Capto MMC using PreDictor plates	28927790
Application note: High-throughput screening of elution pH for monoclonal antibodies on MabSelect SuRe using PreDictor plates	28927792
Application note: Adsorption equilibrium isotherm studies using a high-throughput method	28940362
Application note: High-throughput screening and column optimization of a monoclonal antibody capture step	28940347

Additional literature	Code no.
Data file: Capto S, Capto Q, Capto ViralQ, and Capto DEAE	11002576
Data file: Capto MMC	11003545
Data file: Capto adhere	28907888
Data file: MabSelect	18114994
Data file: MabSelect SuRe	11001165
Data file: MabSelect Xtra	11001157
Data file: Sepharose Fast Flow ion exchangers	18102066
Application note: Screening and optimization of loading conditions on Capto S	28407816
Data file: ÄKTA avant 25	28957345
Data file: UNICORN 6 control software	28-957346
Application note: Rapid process development for purification of a MAb using ÄKTA avant 25	28957347
HiScreen Octyl FF	28926986
HiScreen Phenyl FF (high sub)	28926988
HiScreen Phenyl FF (low sub)	28926989

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