GE Healthcare

Instructions 28-9258-34 AD **High-throughput process development**

PreDictor plates

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1 Introduction

PreDictor™ plates are disposable 96-well filter plates prefilled with GE Healthcare BioProcess™ chromatography media, see Table 1. PreDictor plates support high-throughput process development (HTPD) by allowing parallel screening of chromatographic conditions. They can be used in automated workflows using robotic systems, or can be operated manually using multi-channel pipettes. Each well in a PreDictor plate is prefilled with a defined amount of chromatography medium. The choice of PreDictor plate depends on the type of application, see Table 2 and Section 2.2 for details.

As a result of parallel screening of chromatographic conditions, a large number of experimental conditions may be evaluated simultaneously. This allows screening of a large experimental space to identify the subspace that is the most relevant with respect to one or several defined responses. All chromatography media used in PreDictor plates are available in prepacked columns and in bulk packs. This means that once the experimental subspace has been found, optimization and scale-up can easily be done on columns using ÄKTAdesign™ systems.

Table 1. Available PreDictor plate products

Product	Chromatography medium volume per well[1]
PreDictor Capto™ Q single medium plate	2 µl, 20 µl or 50 µl
PreDictor Capto S single medium plate	2 µl, 20 µl or 50 µl
PreDictor Capto DEAE single medium plate	2 µl, 20 µl or 50 µl
PreDictor Capto MMC single medium plate	6 µl, 20 µl or 50 µl
PreDictor Capto adhere single medium plate	6 µl, 20 µl or 50 µl
PreDictor Q Sepharose™ Fast Flow single medium plate	6 µl, 20 µl or 50 µl
PreDictor SP Sepharose Fast Flow single medium plate	6 µl, 20 µl or 50 µl
PreDictor MabSelect™ single medium plate	6 µl, 20 µl or 50 µl
PreDictor MabSelect SuRe™ single medium plate	6 µl, 20 µl or 50 µl
PreDictor MabSelect Xtra™ single medium plate	6 µl, 20 µl or 50 µl
PreDictor AIEX screening plate[2]	2 µl/6 µl or 20 µl
PreDictor CIEX screening plate[3]	2 µl/6 µl or 20 µl
PreDictor isotherm plate	Different medium volume in different wells (2 µl, 4 µl, 6 µl, 8 µl, 20 µl and 50 µl)

1 Note that the total medium suspension volume per well is larger than the chromatography medium volume. For total medium suspension volume per well, see Table 7.

2 PreDictor AIEX screening plate 2µl/6µl contains following chromatography medium volumes per well: Capto Q 2 µl, Capto DEAE 2 µl, Q Sepharose Fast Flow 6 µl and Capto adhere 6 µl. PreDictor AIEX screening plate 20 µl contains 20 µl per well of the corresponding media.

3 PreDictor CIEX screening plates 2µl/6µl contains following chromatography medium volumes per well: Capto S 2 µl, SP Sepharose Fast Flow 6 µl and Capto MMC 6 µl. PreDictor CIEX screening plate 20 µl contains 20 µl per well of the corresponding media.

2 Applications

PreDictor plates can be used to screen different parts of the chromatographic cycle, for example determination of binding, wash, and elution conditions. It is possible to perform adsorption isotherm studies and time-dependent studies (quantitative or qualitative). Quantitative analysis of very low concentrations of proteins and/or impurities may be limited by non-specific adsorption to the PreDictor plate. Regardless of the application, the workflow includes equilibration, sample addition, incubation, wash, and elution - that is similar to a typical chromatographic cycle in a column.

Related literature

Application specific protocols are described in the related literature listed below, see also Section 7.4:

- Screening of loading conditions on Capto S using a new high-throughput format, PreDictor plates
- High-throughput screening of elution conditions on Capto MMC using PreDictor plates
- High-throughput screening of elution pH for monoclonal antibodies on MabSelect SuRe using PreDictor plates
- Adsorption equilibrium isotherm studies using a high-throughput method
- High-throughput screening and column optimization of a monoclonal antibody capture step

2.1 Batch uptake experiment

In a typical adsorption process, both the mass transfer mechanism responsible for protein transport and the ligand selectivity are independent of the mode of operation (i.e. are the same regardless of whether they occur in a batch system or packed column). If a column is approximated by a cascade of hypothetical stages (theoretical plates) where a separation occurs, a single well in a PreDictor plate can be seen 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. As long as a difference in adsorption capacities/rates for different constituents of a sample can be quantified in a single well, the results obtained using PreDictor plates can be used to describe the same separation occurring in a column.

Fig 1 shows a batch uptake experiment taking place in the wells of the PreDictor plates. The steps in PreDictor plate experiments are the same as in a typical chromatographic separation: equilibration, sample loading, wash and elution.

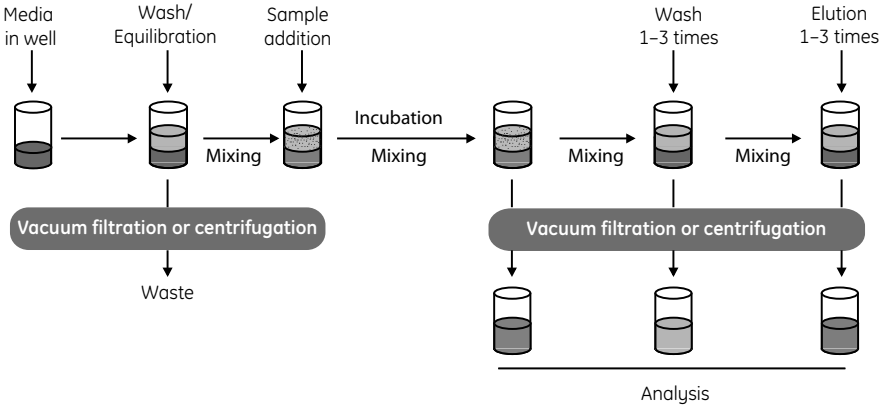


Fig 1. Schematic drawing of a batch uptake experiment taking place in the wells of PreDictor plates.

2.2 PreDictor plate selection

Different PreDictor plates

For optimal results, different applications and samples will require that the correct type of plate is used. Different types of PreDictor plates are therefore available to provide flexibility for designing a study. The plates available can be divided into three main categories:

1 Single medium plates

- For binding, wash or elution studies
- Same medium volume in all wells
- For each medium there are 3 different single medium plates available, each with a different medium volume per well. Select medium volume per well depending on type of study, see Table 2.

2 Media screening plates

- For binding, wash or elution studies on multiple chromatography media
- Two types of plates are available:
  - Anion and multimodal anion exchange media plate (Capto Q, Capto DEAE, Q Sepharose Fast Flow and Capto adhere), see Table 3.
  - Cation and multimodal cation exchange media plate (Capto S, SP Sepharose Fast Flow and Capto MMC), see Table 4.
- Two different media screening plates, each with different media volume per well are available. Select medium volume per well depending on type of study, see Table 2.

3 Adsorption isotherm plates

- For binding studies done under equilibrium conditions to obtain fundamental thermodynamic understanding of the adsorption process.
- Contains a single medium in all wells but with different volume of chromatography medium in different wells, see Table 5.
  The plate design, with different amounts of medium in different wells, allows simple and rapid construction of isotherms since it is possible to use a single sample concentration. For further reading on adsorption isotherms, see handbook: *High-throughput process development with PreDictor plates*, and application note: *Adsorption equilibrium isotherm studies using a high-throughput method* (for ordering see Section 7.4).

Selecting plate type

The type of study, amount of sample, target protein and impurities required for analysis need to be considered when selecting a PreDictor plate.

- For binding studies, generally plates with 2 or 6 µl chromatography medium should be used. The chromatography medium is overloaded with protein and the amount of unbound protein is measured. Alternatively, the amount of bound protein is determined from elution pool(s). The different volumes of chromatography media used are based on the properties of the different chromatography media: for the 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 the first choice is the use of 20 µl plates. Larger medium volumes (50 µl) may be required if sample purity needs to be determined. In such cases, minimum detectable amount of impurities will determine the choice of PreDictor plate.
- Screening plates are provided to facilitate media screening. Instead of using several single medium plates to screen different media, plates containing 3 (PreDictor CIEX screening plate) or 4 (PreDictor AIEX screening plate) different media are available.

  Note: *Multimodal CIEX and multimodal AIEX media are found in CIEX screening plates and AIEX screening plates, respectively. These media generally require that a different experimental space is explored as compared to the traditional ion exchange media.*

- Adsorption isotherm plates are provided to facilitate easy construction of an adsorption isotherm, i.e., obtaining data of capacity as a function of equilibrium concentration. One adsorption isotherm plate is provided per medium.
- If a large amount of sample is needed for analysis, a larger medium volume and/or increased number of sample aliquots are needed. Alternatively, several replicates from one plate can be pooled for analysis.

Table 2. PreDictor plate selection guide.

Single medium plates: Binding conditions								
Medium volume per well (µl)	**Capto Q**	**Capto S**	**Capto DEAE**	**Capto MMC**	**Capto adhere**	**Q Sepharose Fast Flow**	**SP Sepharose Fast Flow**	**Mab-Select family**
2	+ +	+ +	+ +	NA	NA	NA	NA	NA
6	NA	NA	NA	+ +	+ +	++	++	+ +
20	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-

Single medium plates: Wash/elute conditions								
Medium volume per well (µl)	**Capto Q**	**Capto S**	**Capto DEAE**	**Capto MMC**	**Capto adhere**	**Q Sepharose Fast Flow**	**SP Sepharose Fast Flow**	**Mab-Select family**
2	-	-	-	NA	NA	NA	NA	NA
6	NA	NA	NA	-	-	-	-	-
20[1]	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
50[2]	+	+	+	+	+	+	+	+

Screening plates: Binding conditions		
Medium volume per well (µl)	**AIEX screening plate (See Table 3 for details)**	**CIEX screening plate (See Table 4 for details)**
2 or 6	+ +	+ +
20	-	-

Screening plates: Wash/elute conditions		
Medium volume per well (µl)	**AIEX screening plate (See Table 3 for details)**	**CIEX screening plate (See Table 4 for details)**
2 or 6	-	-
20	+ +	+ +

Isotherm plates: for determination of adsorption isotherms	
Medium volume per well (µl):	Different in different wells: 2, 4, 6, 8, 20 and 50, see Table 5 for details.
Media available:	Capto Q, Capto S, Capto DEAE, Capto MMC, Capto adhere, Q Sepharose Fast Flow, SP Sepharose Fast Flow, MabSelect, MabSelect SuRe and MabSelect Xtra. Note: only one type of media per plate

++ First choice
+ Possible
- Not recommended
NA Product not available

1 The 20 µl plate is the preferred plate for the first set of experiments.

2 The 50 µl plate may be used for certain experiments, for example when protein concentrations are in the higher range or when there is a need for high amounts of sample for analysis.

Table 3. Media distribution on AIEX screening plates (2 µl/6 µl or 20 µl medium per well).

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
A	Capto Q (2 µl or 20 µl)			Capto DEAE (2 µl or 20 µl)			Q Sepharose Fast Flow (6 µl or 20 µl)			Capto adhere (6 µl or 20 µl)		
B												
C												
D												
E												
F												
G												
H												

Table 4. Media distribution on CIEX screening plates (2 µl/6 µl or 20 µl medium per well).

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
A	Capto S (2 µl or 20 µl)				SP Sepharose Fast Flow (6 µl or 20 µl)				Capto MMC (6 µl or 20 µl)			
B												
C												
D												
E												
F												
G												
H												

Table 5. Medium volume distribution on isotherm plates (numbers in µl).

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
A	50	50	20	20	8	8	6	6	4	4	2	2
B	50	50	20	20	8	8	6	6	4	4	2	2
C	50	50	20	20	8	8	6	6	4	4	2	2
D	50	50	20	20	8	8	6	6	4	4	2	2
E	50	50	20	20	8	8	6	6	4	4	2	2
F	50	50	20	20	8	8	6	6	4	4	2	2
G	50	50	20	20	8	8	6	6	4	4	2	2
H	50	50	20	20	8	8	6	6	4	4	2	2

3 Characteristics

PreDictor plates are disposable 96-well filter plates, each well prefilled with a defined amount of chromatography medium. The available chromatography media are anion exchangers, cation exchangers, multimodal media, and affinity media for capture of monoclonal antibodies, see Table 1. A barcode facilitates the identification of individual plates. Tables 6 and 7 present available chromatography media and characteristics of PreDictor plates, respectively.

Table 6. Characteristics of chromatography media available in PreDictor plates

Chromatography medium	Characteristics	Matrix
Capto Q	Strong anion exchanger	Highly cross-linked agarose with dextran surface extender
Capto S	Strong cation exchanger	Highly cross-linked agarose with dextran surface extender
Capto DEAE	Weak anion exchanger	Highly cross-linked agarose with dextran surface extender
Capto MMC	Multimodal weak cation exchanger	Highly cross-linked agarose
Capto adhere	Multimodal strong anion exchanger	Highly cross-linked agarose
Q Sepharose Fast Flow	Strong anion exchanger	6% cross-linked agarose
SP Sepharose Fast Flow	Strong cation exchanger	6% cross-linked agarose
MabSelect	Recombinant protein A *(E. coli)*	Highly cross-linked agarose
MabSelect SuRe	Alkali-stabilized protein A-derived ligand *(E. coli)*	Highly cross-linked agarose
MabSelect Xtra	Recombinant protein A *(E. coli)*, high binding capacity	Highly cross-linked agarose

Details of the different chromatography media are found in data files for respective media, see Section 7.4.

Table 7. PreDictor plate characteristics

Plate size	127.8 × 85.5 × 30.6 mm (according to ANSI/SBS 1-2004, 3-2004 & 4-2004 standards)
Plate material	Polypropylene and polyethylene
Number of wells	96
Well volume	800 µl
Working volume/well when incubating on a microplate shaker	100 to 300 µl[1]
Volume sedimented medium/well	2 µl, 6 µl, 20 µl or 50 µl For PreDictor isotherm plates different in different wells: 2, 4, 6, 8, 20 and 50 µl
Medium suspensions in total volume of	• 200 µl for 2 µl sedimented medium/well • 500 µl for 6, 20, and 50 µl sedimented medium/well • For PreDictor isotherm plates: – 500 µl for 50 µl sedimented medium/well – 200 µl for 20 µl sedimented medium/well – 500 µl for 8 µl sedimented medium/well – 375 µl for 6 µl sedimented medium/well – 250 µl for 4 µl sedimented medium/well – 125 µl for 2 µl sedimented medium/well
Storage solution	• PreDictor Capto S, SP Sepharose Fast Flow, CIEX screening, Capto S isotherm and SP Sepharose Fast Flow isotherm: 20% ethanol + 0.2 M sodium acetate • All other PreDictor plates: 20% ethanol
Recommended storage temperature	• PreDictor MabSelect, MabSelect SuRe and MabSelect Xtra: +4°C to +8°C • All other PreDictor plates: +4°C to +30°C
Working temperature	+4°C to +30°C
Centrifugation force **recommended** **maximum**	 300 to 500 × *g* (Sample dependent) 700 × *g*
Vacuum **recommended** **maximum**	 -0.15 to -0.3 bar (Sample dependent) -0.5 bar
Microplate shaker shaking speed	1100 rpm with 3 mm circular centripetal movement or sufficient mixing to maintain slurried chromatography medium in wells.
Barcode	Placed on one of the short ends of the PreDictor plate and containing: • Article number • Lot number • Individual identification number

[1] The lower volume in this interval indicates the working volume needed for effective mixing of sample/liquid on microplate shaker. The upper limit is the limiting volume for avoiding cross contamination between wells during mixing on a microplate shaker without sealing the top of the PreDictor plate.
Note: The volume and the amount of protein needed for analysis are also to be taken into consideration.

4 Advice on handling

4.1 Equipment

PreDictor plates are designed for both manual and robotic handling. Table 8 is a guide to the equipment required for manual and robotic handling of PreDictor plates.

For an automated workflow, using robotic handling, note that following items are required:

- an automated blotting device to avoid leakage and contamination
- an automated microplate shaker with holding devices to keep the collection plate in place when mixing

Table 8. Recommended equipment for manual and robotic handling of PreDictor plates

Equipment	Details	Tips and tricks
Pipette	Use an 8 or 12 multi-channel pipette for quick and easy pipetting of liquids into the PreDictor plates.	When dispensing liquid it is useful to aspirate a larger volume and thereafter dispense the liquid into the PreDictor plate wells in smaller fixed volumes in several steps.
Collection plate	Use a 96-well microplate (UV- or non-UV readable).	To avoid overfilling the collection plate, make sure not to add a larger volume to the wells of the PreDictor plate than the volume of the wells in the collection plate. When the collection plate is to be frozen, do not fill the wells to more than half of the handling volume. When using a UV readable collection plate, make sure not to touch the bottom of the collection plate.
Microplate shaker	Use a microplate shaker with 3 mm circular centripetal movement and regulation speed of 1100 rpm to fully suspend the sample/buffer in the medium during incubation.	Safely secure the PreDictor plate and the collection plate on the microplate shaker. For example, use a rubber band to secure the plates to each other.
Centrifuge	Use a swing-out rotor with microplate carriers capable of handling a PreDictor plate on top of a collection plate (for PreDictor plate size, see Table 7).	Centrifuge within 300–500 × *g* (max 700 × *g*) for 1 min or until all liquid is removed. If liquid is left in the wells after centrifugation, increase the speed (max 700 × *g*) and centrifuge for another 1 min.

Equipment	Details	Tips and tricks
Vacuum manifold	Designed and optimized for vacuum filtration of 96-well PreDictor plates (for PreDictor plate size, see Table 7).	The distance between the bottom of the PreDictor plate and the top of the collection plate in the vacuum manifold should be about 5 mm to avoid cross contamination in the collection plate during vacuum filtration. Place an appropriate spacer block into the lower chamber of the vacuum manifold to reduce the distance between the plates. Place the PreDictor plate on the vacuum manifold. Set the vacuum within -0.15 to -0.5 bar. Apply vacuum until all solution is removed.
Reagent reservoir	Use an 8-, 48- or 96-well deep well reservoir for buffer/solution preparation.	Prepare a separate 48- or 96-well deep well plate with the appropriate solutions in order to facilitate the transfer of solutions according to the experimental plan. Seal the deep well plate filled with prepared solutions with an appropriate plate seal or sealing tape to reuse the solutions.
	Use a reagent reservoir with v-shaped bottom for buffer/solution preparation. (Manual handling)	Use a reagent reservoir with a v-shaped bottom to allow easy withdrawal of solution and to minimize the volume of liquid needed for pipetting. When pipetting the same buffer/solution in the whole PreDictor plate, use a reagent reservoir filled with solution.
Blotting tissue	Use a soft paper tissue.	To remove drops of liquid that may have accumulated on the bottom of the PreDictor plate, blot the bottom of the PreDictor plate after centrifugation/vacuum filtration in the last equilibration step before sample loading. Blotting can be added in other steps as well. Blotting is important to minimize the risk of leakage of liquid through the filter in the plate.

4.2 Sample preparation

We recommend applying a clarified sample to PreDictor plates, since unclarified sample may cause clogging of the filters in the bottom of the wells. Include centrifugation and/or filtration steps after mechanical and/or chemical lysation of the sample.

4.3 Working with aqueous solutions containing detergents

PreDictor plates are compatible with all aqueous solutions commonly used in purification of biopharmaceuticals. With solutions containing detergents it should be emphasized that some detergents may induce leakage of liquid through the filter in the PreDictor plate. The probability of leakage increases when using detergents with low surface tension. In general, the number of times the detergent passes through the filter in the PreDictor plate should be minimized to avoid leakage through the filter.

Recommendations to minimize leakage when working with detergents

- Avoid use of detergent in equilibration buffer and preferably also in the sample, especially when loading multiple aliquots.
- If detergents must be included in the equilibration buffer and/or in the sample, add it only to the last equilibration step and avoid incubating the sample longer than 1.5 h.
- Minimize the number of sample loadings by carefully choosing a PreDictor plate with appropriate medium volume, see Table 2.
- In cases of persistent leakage, consider using a different detergent.

4.4 Experimental setup

PreDictor plates are designed for efficient screening. When using the high-throughput process development (HTPD) approach in PreDictor plates, it is therefore suggested to screen a broader range of process parameters than usually is done when working with columns.

By using Design of Experiments (DoE) for the experimental set-up, many different chromatographic conditions (factors) can be efficiently screened simultaneously in PreDictor plates. DoE employs statistics to identify and define the factors having the greatest impact on the process/product. For experimental set-up and data evaluation the software Assist is recommended, see Section 4.6.

Examples of conditions to be screened

- pH
- Conductivity/ionic strength
- Salt type
- Buffer species
- Additives

HTPD workflow increases the number of samples to analyze. One plate produces at least 96 samples for analysis. Consider suitable analytical methods, for example UV absorbance, ELISA, Biacore™ based assays (real time SPR), etc.

One product package containing 4 PreDictor plates is sufficient to perform for example 128 runs in a study using triplicates. We recommend replicates to allow for outlier analysis. For larger studies, preferably use PreDictor plates from the same lot.

Examples of experimental set-ups are described in PreDictor plate application notes, see PreDictor plate literature, Section 7.4.

4.5 Sample incubation time

Sample incubation time for most studies is 30 to 60 min. With adsorption isotherm plates longer incubation times are needed, 2 to 6 h as data under equilibrium conditions are to be collected. If the effect of incubation time is to be studied a time range of 2 to 60 min is recommended.

The reason for apparently long incubation times in plates as compared to residence time in column chromatography relates to the differences in the techniques. The incubation time corresponds better to the loading time in columns since this reflects to total time the medium particles are in contact with the sample, see handbook, *Working with PreDictor plates*, for details.

4.6 Assist software

Assist software is designed to support the HTPD workflow using PreDictor plates from set up of experimental design to data evaluation, see Fig 2.

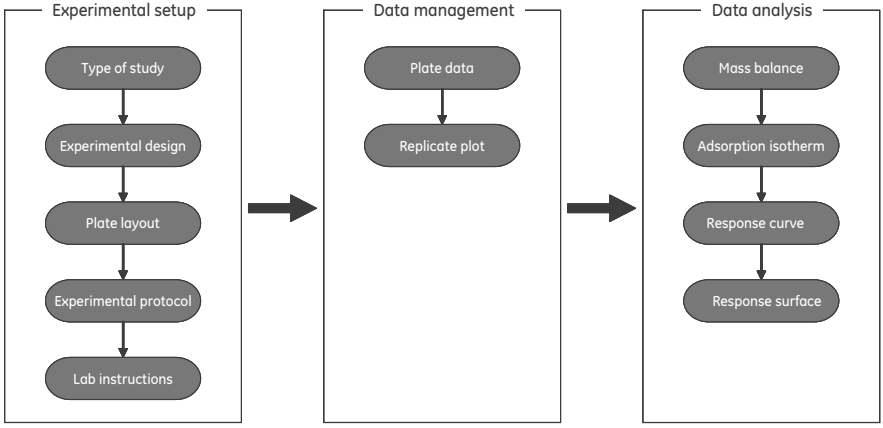


Fig 2. Assist software support to the HTPD workflow using PreDictor plates.

Experimental set up
The experimental design is set by defining variation of experimental conditions such as buffer system, pH and salt concentration. The user enters this information to the Assist software which suggests an experimental design. The software generates one or more plate layouts of which the user selects one. The plate layout defines the distribution of experimental conditions across wells. Documentation of the selected experimental design, such as protocol and lab instructions is also generated.

Data management
After the experiment has been performed it is possible to load plate data, view replicates and exclude outliers.

Data analysis
In data analysis it is possible to calculate and visualize mass balance, adsorption isotherms, response surfaces and response curves. Data analysis will show how experimental conditions affect yield, binding capacity, recovery etc.

5 Protocol

The protocol is designed as a general guideline for working with PreDictor plates. Optimization may be required depending on sample, type of study, and chromatography medium volume in wells. The PreDictor plates can be operated manually by using a multi-channel pipette or in robotic systems. Removal of liquid can be performed either by centrifugation or vacuum filtration.

There is an instruction video, *Learn more about how to work with PreDictor plates*, available at: *www.gelifesciences.com/predictor*

5.1 General considerations

Automated operation

The protocol (Section 5.2) refers to manual operation. For automated operation using a robotic system, make sure that the robot is adequately equipped to support the individual steps in the protocol.

Opening PreDictor plates

It is important to carefully follow instructions for steps 1 and 2 in the protocol (Section 5.2). If not followed there is a risk that chromatography medium remains attached to the top seal.

Leakage

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. Always keep the PreDictor plate on a collection plate, see Section 7.3, or on an other appropriate spacer throughout the workflow.
- blot the bottom of the PreDictor plate on a soft paper tissue after centrifugation or vacuum filtration in the last equilibration step before sample loading.
  After blotting, the PreDictor plate must be put on a collection plate (Section 7.3) or an other appropriate spacer before further operation.
- ensure that the PreDictor plate and the collection plate are fixed to each other during mixing (see *Mixing* below). If the PreDictor plate outlets (the drips) rub against the edges of the collection plate wells, leakage may occur.

Contamination

- Always put the PreDictor plate on a collection plate (Section 7.3) or other spacer to minimize risk of contamination.
- Avoid putting the PreDictor plate directly on the lab bench or other surface.

Evaporation

To reduce evaporation effects when using incubation times longer than 1 hour, consider to cover the PreDictor plate using a self-adhesive microplate foil (see Section 7.3) or an other appropriate 96-well cover.

Mixing

The PreDictor plate and the collection plate must be fixed to each other and to the microplate shaker during mixing. If the PreDictor plate outlets (the drips) rub against the edges of the collection plate wells, leakage may occur. For example, use a rubber band to secure the plates to each other and to the microplate shaker.

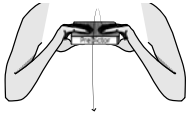
Sample and solution addition to PreDictor plates

In order to minimize loading generated artefacts, add samples, buffers and solutions to the whole PreDictor plate without delay.

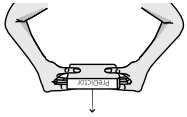
5.2 Detailed protocol

1 Resuspend the medium (20x)

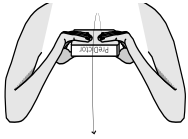
To resuspend medium particles attached to the top seal, shake PreDictor plates as described (step 1A to 1D).



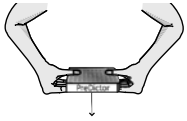
A Hold the PreDictor plate (top side up) with both hands. Keep the thumbs on the bottom side of the PreDictor plate and the other fingers on the top side.
Rotate the PreDictor plate to bottom side up while thrusting it downwards in a swift, controlled movement until the arms are fully extended.



B Finish the movement with a flick downwards.



C Reposition hands to hold thumbs under the PreDictor plate and the other fingers over (as above, but now with PreDictor plate bottom up). Repeat the rotation, making the top side up again.

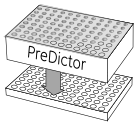


D Finish the movement with a flick downwards.

Repeat the rotations (step 1A to 1D) 20 times (10 times for each side).

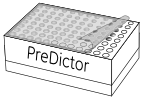
2 Remove cover seals

A Hold the PreDictor plate horizontally and peel off the bottom seal.



B Place the PreDictor plate on a collection plate.

C Let the PreDictor plate rest for at least 1 minute to allow slurried medium to slide down from the well walls.



D Gently peel off the top seal from the PreDictor plate while holding it against the collection plate.

3 Remove storage solution

Note: *Remember to change or empty the collection plate, when necessary during the following steps.*

- Centrifuge the plates for 1 minute at 500 × g, or until all storage solution is removed.

or

- Remove storage solution by vacuum filtration:

  Place the collection plate into the lower chamber of the vacuum manifold.

  Turn on the vacuum (-0.15 to -0.5 bar) and then place the PreDictor plate on the vacuum manifold.

  Turn off the vacuum as soon as all solution is removed, to avoid cross contamination in the collection plate.

  Note: *The distance between the bottom of the PreDictor plate and the top of the collection plate in the vacuum manifold should be about 5 mm to avoid cross contamination in the collection plate. Place an appropriate spacer block into the lower chamber of the vacuum manifold to reduce the distance between the plates.*

4 Equilibrate (3×)

A Add 200 µl equilibration buffer/well.

B Mix briefly on a microplate shaker at 1100 rpm (e.g. 1 minute). Fix the PreDictor plate and the collection plate to each other and secure them to the microplate shaker during mixing. The mixing will increase the efficiency of the equilibration.

C Remove equilibration buffer by:

- centrifugation for 1 minute at 500 × g or until all solution is removed.

or

- vacuum filtration, as described in step 3.

Perform the equilibration step at least three times or until the medium is equilibrated.

5 Blot

A After centrifugation or vacuum filtration in *the last equilibration step,* blot the bottom of the PreDictor plate on a soft paper tissue to remove drops of equilibration buffer that may have accumulated on the bottom of the PreDictor plate.

B After blotting, always place the PreDictor plate on a collection plate before further operation.

Note: *Blotting is important to minimize risk of leakage of liquid through the filter in the PreDictor plate, thus to obtain good quality results. Blotting may be added in other steps as well.*

6 Load sample

A Apply 100 to 300 µl clarified sample per well.
Larger sample volumes can be loaded in aliquots. Maximum number of recommended aliquots is 3.

Note: *Minimize the number of aliquot loadings by choosing a PreDictor plate with appropriate medium volume, see Table 2.*

B Incubate on a microplate shaker at 1100 rpm.

Fix the PreDictor plate and the collection plate to each other and secure them to the microplate shaker during mixing.

The top of the PreDictor plate may be covered by use of a microplate foil (see Section 7.2) or an appropriate 96-well cover.

Note: *Incubation time is application related (see Section 4.5 or Section 7.4 for related literature).*
Incubation time for most studies is 30 to 60 min. With adsorption isotherm plates incubation times of 2 to 6 h are required. If the effect of incubation time is to be studied a time range of 2 to 60 min is recommended.

C Remove supernatant by:

- centrifugation for 1 minute at 500 × g or until all solution is removed. Centrifugation force and/or time may require adjustment.
  If covering the top of the PreDictor plate, remove the cover before centrifugation.

or

- vacuum filtration, as described in step 3.

7 Wash out unbound sample (3×)

A Add 200 µl equilibration buffer/well.

B Mix briefly on a microplate shaker at 1100 rpm (e.g. 1 minute). Fix the PreDictor plate and the collection plate to each other and secure them to the microplate shaker during mixing. The mixing will increase the efficiency of the wash.

C Remove unbound sample by:

- centrifugation for 1 minute at 500 × g.

or

- vacuum filtration, as described in step 3.

Three wash steps are typically sufficient to remove all unbound sample. Remember to change/empty the collection plate between each wash step.

- **Optional: Intermediate wash (1-3×)**
  Intermediate wash solutions may be introduced in this optional step.

A Add 200 µl of desired wash buffer/well.

B Follow step 7B to 7C with either centrifugation or vacuum filtration supernatant removal. Remember to change/empty the collection plate between each intermediate step.

8 Elute (3×)

A Add 200 µl of elution buffer/well.

B Mix briefly on a microplate shaker at 1100 rpm.
Fix the PreDictor plate and the collection plate to each other and secure them to the microplate shaker during mixing. The mixing will increase the efficiency of the elution.

C Elute sample by:

- Centrifuge for 1 minute at 500 × g

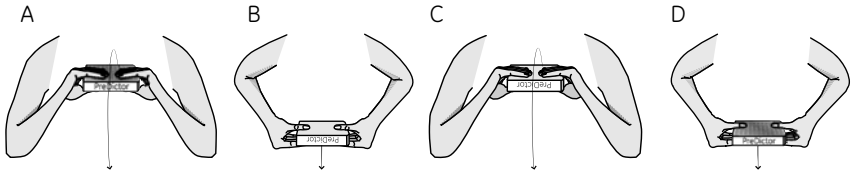
or

- vacuum filtration, as described in step 3.

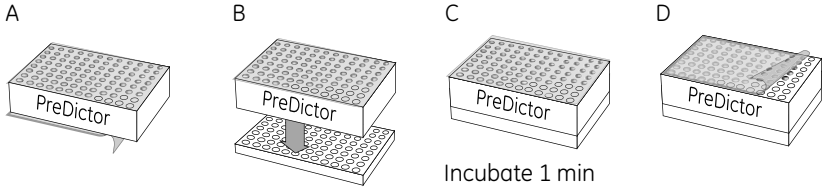
Three elution steps are typically sufficient to elute the sample. Remember to change collection plates between each elution step.

5.3 Protocol quick guide

1 Resuspend the medium (20x: 10 times for each side)



2 Remove cover seals



Incubate 1 min

3 Remove storage solution

Centrifuge 1 min at 500 × g or Vacuum filtrate until all solution is removed

4 Equilibrate (3×)

Add 200 µl equilibration buffer/well

Mix briefly on a microplate shaker at 1100 rpm

Centrifuge 1 min at 500 × g or Vacuum filtrate until all solution is removed

5 Blot


Blot the bottom of the PreDictor plate on a soft paper tissue

6 Load sample (3×)

Load 100 to 300 µl clarified sample per well

Mix briefly on a microplate shaker at 1100 rpm

Centrifuge 1 min at 500 × g or Vacuum filtrate until all solution is removed

7 Wash out unbound sample (3×)

Add 200 µl equilibration buffer/well

Mix briefly on a microplate shaker at 1100 rpm

Centrifuge 1 min at 500 × g or  Vacuum filtrate until all solution is removed

8 Elute (3x)

Add 200 µl of elution buffer/well

Mix briefly on a microplate shaker at 1100 rpm

Centrifuge 1 min at 500 × g or  Vacuum filtrate until all solution is removed

Note: *Remember to change collection plates between each elution step.*

6 Troubleshooting guide

Fault	Possible cause	Action
PreDictor plate wells are clogged.	• The sample is too viscous.	• Increase dilution of the cell paste before lysis, or dilute after the lysation.
	• There is too much cell debris in the sample.	• Centrifuge and/or filtrate the sample if unclarified sample has been used.
Problem with reproducibility and/or cross contamination in the collection plate when using vacuum filtration.	• The vacuum is too high or too low.	• Decrease or increase the vacuum.
	• The distance between the PreDictor plate and the collection plate is too large or too small.	• Reduce or increase the distance between the PreDictor plate and the collection plate during vacuum filtration. The distance between the bottom of the PreDictor plate and the top of the collection plate in the vacuum manifold should be about 5 mm to avoid cross contamination. Place an appropriate spacer block into the lower chamber of the vacuum manifold to reduce the distance between the plates.
	• The rubber gasket in the vacuum manifold is worn out.	• Make sure that the rubber gasket in the vacuum manifold tightens around the PreDictor plate. All wells should be emptied simultaneously.
		• If the problem still occurs, change to centrifugation. When using centrifugation, different centrifugation forces may be tried (within the interval 300–500 × g, max 700 × g, for 1 min).
Problem with foam in the collection plate when using vacuum.	• The vacuum is too high.	• Decrease the vacuum.
	• The time it takes to empty the wells is too long.	• Empty the wells more rapidly. The wells should be emptied as fast as possible. Turn off the vacuum as soon as the wells are empty. Vacuum filtration time at -0.5 bar is about 10 seconds.
	• The sample is too viscous.	• Reduce the sample viscosity

Fault	Possible cause	Action
	• The protein concentration is too high.	• Reduce the protein concentration and/or use a PreDictor plate with another medium volume (see Table 2).
Problem with leakage through the filter in the PreDictor plate during sample incubation	• The PreDictor plate is not placed on a collection plate.	• During all handling of the PreDictor plate when the bottom seal is not present, always put it on a collection plate to minimize risk of leakage through the filter.
	• Drops of equilibration buffer have accumulated on the bottom of the PreDictor plate.	• Blot the bottom of the PreDictor plate on a soft paper tissue after centrifugation/vacuum filtration in the last equilibration step before sample loading. Blotting may be added in other steps as well. This is important in order to minimize risk of leakage of liquid through the filter in the plate during incubation.
	• Sample has been loaded too many times.	• Maximum number of recommended aliquots is 3. Too many aliquots may result in leakage through the filter in the PreDictor plate, and is also time consuming.
	• The PreDictor plate and the collection plate are not fixed to each other during mixing on the microplate shaker. If filter outlets (the drips) rub against the edges of the collection plate wells, leakage may occur.	• Safely secure the PreDictor plate and the collection plate on the microplate shaker. The plates must also be fixed to each other. For example, use a rubber band to secure the plates to each other.
	• Detergent is included in equilibration buffer and/or sample.	• Perform the equilibration if possible without detergents in the buffer. If detergent must be included in the equilibration buffer, add it only to the last equilibration step and incubate the sample no longer than 1.5 h. In cases of persistent leakage, consider using a different detergent.
	• The PreDictor plate has been used in previous experiments.	• The PreDictor plate is a disposable item. Always use new PreDictor plates when setting up new experiments.

7 Ordering information

For information about related products, accessories, and related literature, see online information at: *www.gelifescience.com/predictor*

7.1 PreDictor plates

Single medium plates	No. supplied	Code no.
PreDictor Capto Q, 2 µl	4 × 96-well filter plates	28-9257-73
PreDictor Capto Q, 20 µl	4 × 96-well filter plates	28-9258-06
PreDictor Capto Q, 50 µl	4 × 96-well filter plates	28-9258-07
PreDictor Capto S, 2 µl	4 × 96-well filter plates	28-9258-08
PreDictor Capto S, 20 µl	4 × 96-well filter plates	28-9258-09
PreDictor Capto S, 50 µl	4 × 96-well filter plates	28-9258-10
PreDictor Capto DEAE, 2 µl	4 × 96-well filter plates	28-9258-11
PreDictor Capto DEAE, 20 µl	4 × 96-well filter plates	28-9258-12
PreDictor Capto DEAE, 50 µl	4 × 96-well filter plates	28-9258-13
PreDictor Capto MMC, 6 µl	4 × 96-well filter plates	28-9258-14
PreDictor Capto MMC, 20 µl	4 × 96-well filter plates	28-9258-15
PreDictor Capto MMC, 50 µl	4 × 96-well filter plates	28-9258-16
PreDictor Capto adhere, 6 µl	4 × 96-well filter plates	28-9258-17
PreDictor Capto adhere, 20 µl	4 × 96-well filter plates	28-9258-18
PreDictor Capto adhere, 50 µl	4 × 96-well filter plates	28-9258-19
PreDictor MabSelect, 6 µl	4 × 96-well filter plates	28-9258-20
PreDictor MabSelect, 20 µl	4 × 96-well filter plates	28-9258-21
PreDictor MabSelect, 50 µl	4 × 96-well filter plates	28-9258-22
PreDictor MabSelect SuRe, 6 µl	4 × 96-well filter plates	28-9258-23
PreDictor MabSelect SuRe, 20 µl	4 × 96-well filter plates	28-9258-24
PreDictor MabSelect SuRe, 50 µl	4 × 96-well filter plates	28-9258-25
PreDictor MabSelect Xtra, 6 µl	4 × 96-well filter plates	28-9432-75
PreDictor MabSelect Xtra, 20 µl	4 × 96-well filter plates	28-9432-76
PreDictor MabSelect Xtra, 50 µl	4 × 96-well filter plates	28-9432-77
PreDictor Q Sepharose Fast Flow, 6 µl	4 × 96-well filter plates	28-9432-69
PreDictor Q Sepharose Fast Flow, 20 µl	4 × 96-well filter plates	28-9432-70
PreDictor Q Sepharose Fast Flow, 50 µl	4 × 96-well filter plates	28-9432-71
PreDictor SP Sepharose Fast Flow, 6 µl	4 × 96-well filter plates	28-9432-72
PreDictor SP Sepharose Fast Flow, 20 µl	4 × 96-well filter plates	28-9432-73
PreDictor SP Sepharose Fast Flow, 50 µl	4 × 96-well filter plates	28-9432-74

Screening plates	No. supplied	Code no.
PreDictor AIEX screening (2 µl/6 µl)	4 × 96-well filter plates	28-9432-88
PreDictor AIEX screening (20 µl)	4 × 96-well filter plates	28-9432-89
PreDictor CIEX screening (2 µl/6 µl)	4 × 96-well filter plates	28-9432-90
PreDictor CIEX screening (20 µl)	4 × 96-well filter plates	28-9432-91

Adsorption isotherm plates	No. supplied	Code no.
PreDictor Capto Q isotherm	4 × 96-well filter plates	28-9432-78[1]
PreDictor Capto S isotherm	4 × 96-well filter plates	28-9432-79[1]
PreDictor Capto DEAE isotherm	4 × 96-well filter plates	28-9432-80[1]
PreDictor Capto MMC isotherm	4 × 96-well filter plates	28-9432-81[1]
PreDictor Capto adhere isotherm	4 × 96-well filter plates	28-9432-82[1]
PreDictor MabSelect isotherm	4 × 96-well filter plates	28-9432-83[1]
PreDictor MabSelect SuRe isotherm	4 × 96-well filter plates	28-9432-84[1]
PreDictor MabSelect Xtra isotherm	4 × 96-well filter plates	28-9432-85[1]
PreDictor Q Sepharose Fast Flow isotherm	4 × 96-well filter plates	28-9432-86[1]
PreDictor SP Sepharose Fast Flow isotherm	4 × 96-well filter plates	28-9432-87[1]

[1] Plates are manufactured on request.

7.2 Assist software

Software	Code no.
Assist 1.0 Software package	28-9453-96
Assist, 1-User License 1.0	28-9453-97

7.3 Related products

Accessories	No. supplied	Code no.
Collection plate 96-well 500 µl V-shaped bottom (not UV-readable)	5 × 96 well plates	28-4039-43
Microplate Foil (96-well)	100 × self-adhesive, transparent plastic foils	BR-1005-78

Prepacked columns	No. supplied	Code no.
HiScreen™ Capto Q	1 × 4.7 ml	28-9269-78
HiScreen Capto S	1 × 4.7 ml	28-9269-79
HiScreen Capto DEAE	1 × 4.7 ml	28-9269-82
HiScreen Capto MMC	1 × 4.7 ml	28-9269-80
HiScreen Capto adhere	1 × 4.7 ml	28-9269-81
HiScreen MabSelect	1 × 4.7 ml	28-9269-73
HiScreen MabSelect SuRe	1 × 4.7 ml	28-9269-77
HiScreen MabSelect Xtra	1 × 4.7 ml	28-9269-76

7.4 Related literature

PreDictor literature	Code no.
Handbook: High-throughput process development with PreDictor plates	28-9403-58
Data file: PreDictor 96-well filter plates	28-9258-39
Application note: Screening of loading conditions on Capto S using a new high-throughput format, PreDictor plates	28-9258-40
Mini-poster: High-throughput screening of elution conditions on Capto MMC using PreDictor plates	28-9277-90
Application note: High-throughput screening of elution pH for monoclonal antibodies on MabSelect SuRe using PreDictor plates	28-9277-92
Application note: Adsorption equilibrium isotherm studies using a high-throughput method	28-9403-62
Application note: High-throughput screening and column optimization of a monoclonal antibody capture step	28-9403-47

Literature on related products	Code no.
Data file: Capto S, Capto Q, Capto ViralQ and Capto DEAE	11-0025-76
Data file: Capto MMC	11-0035-45
Data file: Capto adhere	28-9078-88
Data file: MabSelect	18-1149-94
Data file: MabSelect SuRe	11-0011-65
Data file: MabSelect Xtra	11-0011-57
Data file: Sepharose Fast Flow ion exchangers	18-1020-66
Instructions/protocol: Capto S, Capto Q, Capto ViralQ and Capto DEAE	28-4074-52
Instructions/protocol: Capto MMC	11-0035-05
Instructions/protocol: Capto adhere	28-9064-05
Instructions/protocol: MabSelect	71-5020-91
Instructions/protocol: MabSelect SuRe	11-0026-01
Instructions/protocol: MabSelect Xtra	11-0026-02
Instructions/protocol: Sepharose Fast Flow ion exchangers	71-5009-64

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28-9258-34 AD 11/2008