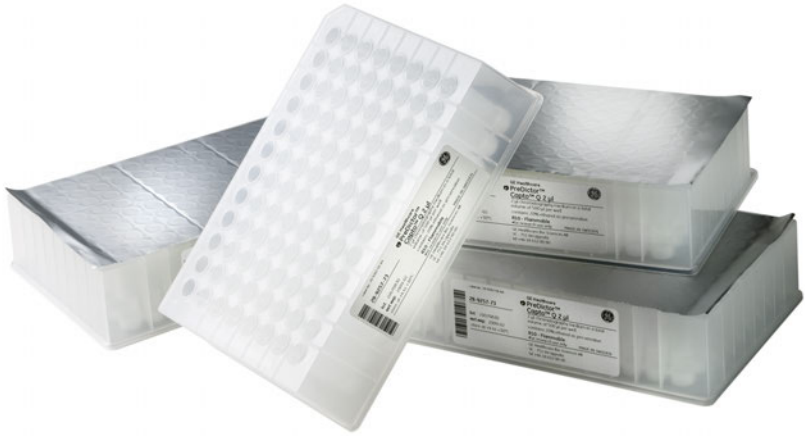
PreDictor™ plates



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Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Introduction

PreDictor plates are disposable 96-well filter plates prefilled with GE Healthcare Life Sciences BioProcess™ chromatography resins, see *Table 1.1*. 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 resin. The choice of PreDictor plate depends on the type of application, see *Table 3.1*, *Table 3.2* and *Chapter 3 PreDictor plate selection, on page 7* 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 resins used in PreDictor plates are available in prepacked columns, such as HiTrap™ and HiScreen™ 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 ÄKTA™ systems.

Table 1.1: Available PreDictor plate products

Product	Chromatography resin volume per well [1]
Single resin plates	
Cation exchange chromatography resins	
PreDictor Capto™ S ImpAct	2 µL or 20 µL
PreDictor Capto SP ImpRes	6 µL or 20 µL
Anion exchange chromatography resins	
PreDictor Capto Q ImpRes	6 µL or 20 µL
PreDictor Capto Q	2 µL, 20 µL or 50 µL
PreDictor Capto DEAE	2 µL, 20 µL or 50 µL
Multimodal chromatography resins	
PreDictor Capto MMC ImpRes	6 µL, 20 µL or 50 µL
PreDictor Capto MMC	6 µL or 20 µL
PreDictor Capto adhere ImpRes	6 µL, 20 µL or 50 µL
PreDictor Capto adhere	6 µL or 20 µL

Product	Chromatography resin volume per well [1]
Affinity chromatography resins	
PreDictor MabSelect™ PrismA	6 µL, 20 µL or 50 µL
PreDictor MabSelect Xtra™	6 µL, 20 µL or 50 µL
PreDictor MabSelect SuRe™	6 µL, 20 µL or 50 µL
PreDictor MabSelect SuRe LX	6 µL, 20 µL or 50 µL
PreDictor MabSelect	6 µL, 20 µL or 50 µL
PreDictor Capto L	6 µL, 20 µL or 50 µL
Hydrophobic interaction chromatography resins	
PreDictor Capto Butyl	6 µL or 50 µL
PreDictor Capto Octyl	6 µL or 50 µL
PreDictor Capto Phenyl (high sub)	6 µL or 50 µL
Screening plates	
PreDictor Capto CIEX polishing screening [2]	2 µL/6 µL or 20 µL
PreDictor CIEX screening plate [3]	2 µL/6 µL or 20 µL
PreDictor Capto AIEX polishing screening [4]	2 µL/6 µL or 20 µL
PreDictor AIEX screening plate [5]	6 µL or 50 µL
PreDictor Capto HIC screening [6]	50 µL
Isotherm plates	
PreDictor Adsorption isotherm plates [7]	Different resin volume in different wells (2 µL, 4 µL, 6 µL, 8 µL, 20 µL, and 50 µL)

1 Note that the total resin suspension volume per well is larger than the chromatography resin volume. For total resin suspension volume per well, see *Table 4.2*.

2 PreDictor Capto CIEX polishing screening plate 2 µL/6 µL contains the following chromatography resin volumes per well: Capto S ImpAct 2 µL, Capto SP ImpRes 6 µL and Capto MMC ImpRes 6 µL. PreDictor Capto CIEX polishing screening plate 20 µL contains 20 µL per well of the corresponding resin.

3 PreDictor CIEX screening plate 2µL/6µL contains the following chromatography resins 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 resin.

4 PreDictor Capto AIEX polishing screening plate 2 µL/6 µL contains the following chromatography resin volumes per well: Capto Q 2 µL, Capto Q ImpRes 6 µL, Capto adhere 6 µL and Capto adhere ImpRes 6 µL. PreDictor Capto AIEX polishing screening plate 20 µL contains 20 µL per well of the corresponding resin.

5 PreDictor AIEX screening plate 2 µL/6 µL contains the following chromatography resin 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 resins.

6 PreDictor Capto HIC screening contains the following chromatography resins: Capto Phenyl (high sub), Capto Phenyl ImpRes, Capto Butyl, Capto Butyl ImpRes, and Capto Octyl.

7 PreDictor Adsorption isotherm plates include 11 different versions, see *Table 3.6* and *Chapter 8 Ordering information, on page 32*.

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, also refer to *Related literature, on page 35*:

- 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
- High-throughput screening of HIC media in PreDictor plates for capturing recombinant Green Fluorescent Protein from *E. coli*
- High-throughput screening and process development for capture of recombinant proinsulin from *E. coli*
- High-throughput process development for design of cleaning-in-place protocols

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 2.1 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.

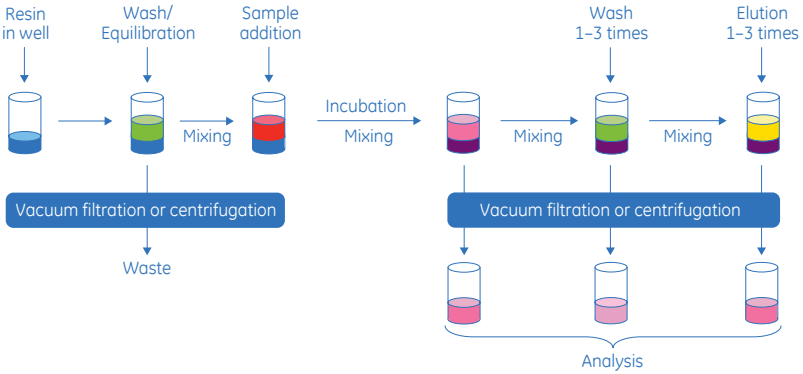


Figure 2.1: Schematic drawing of a batch uptake experiment taking place in the wells of PreDictor plates.

3 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 chromatography resin plates**

- For binding, wash or elution studies.
- Same chromatography resin volume in all wells.
- Different single resin plates are available, each with a different resin volume per well. Select resin volume per well depending on type of study, see *Table 3.1*.

2 **Chromatography resin screening plates**

- For binding, wash or elution studies on multiple chromatography resins.
- Six types of plates are available:
  - Capto CIEX polishing screening resin plate (Capto S ImpAct, Capto SP ImpRes and Capto MMC ImpRes), see *Table 3.2*.
  - Capto AIEX polishing screening resin plate (Capto Q, Capto Q ImpRes, Capto adhere and Capto adhere ImpRes), see *Table 3.2*.
  - Anion screening resin plate (Capto Q, Capto DEAE, Q Sepharose Fast Flow and Capto adhere), see *Table 3.3*.
  - Cation screening resin plate (Capto S, SP Sepharose Fast Flow and Capto MMC), see *Table 3.4*.
  - Capto HIC screening plate (Capto Phenyl (high sub), Capto Phenyl ImpRes, Capto Butyl, Capto Butyl ImpRes, and Capto Octyl), see *Table 3.5*.

- Screening plates are available in different resin volume per well. Select resin volume per well depending on type of study, see *Table 3.1*.

3 **Adsorption isotherm plates**

- For binding studies done under equilibrium conditions to obtain fundamental thermodynamic understanding of the adsorption process.
- Contains a single chromatography resin in all wells but with different volumes of chromatography resin in different wells, see *Table 3.6*.

  The plate design, with different amounts of resin 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, refer to handbook: High-throughput process development with PreDictor plates, and application note:

  Adsorption equilibrium isotherm studies using a high-throughput method (for ordering see *Chapter 8 Ordering information, on page 32*).

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 resin should be used. The resin 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 resin used are based on the properties of the different resins: for the high-capacity ion exchangers 2 µL is sufficient, while for the other resins 6 µL is required for optimal results.
- For wash and elution studies the first choice is the use of 20 µL plates. Larger resin 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 resin screening. Instead of using several single resin plates to screen different resins, plates containing 3 different resins (PreDictor Capto CIEX polishing screening plate and PreDictor CIEX screening plate) or 4 different resins (PreDictor Capto AIEX polishing screening plate, PreDictor AIEX screening plate, and PreDictor Capto HIC screening plate are available.
  Note:
  Multimodal CIEX and multimodal AIEX resins are found in CIEX screening plates and AIEX screening plates, respectively. These resins generally require that a different experimental space is explored as compared to the traditional ion exchange resins.
- 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 chromatography resin.
- If a large amount of sample is needed for analysis, a larger resin volume and/or increased number of sample aliquots are needed. Alternatively, several replicates from one plate can be pooled for analysis.

PreDictor plate selection guide

Table 3.1: Single resin plates for some of the most commonly used resins: Binding and wash/elute conditions

Chromatography resin	Binding conditions (µL/ well)				Wash/elute conditions (µL/ well)			
	2	6	20	50	2	6	20[1]	50[2]
Capto Q	++	NA	-	-	-	NA	++	+
Capto S ImpAct	++	NA	-	NA	-	NA	++	NA
Capto Q ImpRes	NA	++	-	NA	NA	-	++	NA
Capto SP ImpRes	NA	++	-	NA	NA	-	++	NA
Capto DEAE	++	NA	-	-	-	NA	++	+
Capto MMC	NA	++	-	-	NA	-	++	+
Capto MMC ImpRes	NA	++	-	NA	NA	-	++	NA
Capto adhere	NA	++	-	-	NA	-	++	+
Capto adhere ImpRes	NA	++	-	NA	NA	-	++	NA
MabSelect family	NA	++	-	-	NA	-	++	+
Capto L	NA	++	-	-	NA	-	++	+
Capto Butyl	NA	++	NA	-	NA	-	NA	++
Capto Octyl	NA	++	NA	-	NA	-	NA	++
Capto Phenyl (high sub)	NA	++	NA	-	NA	-	NA	++

++	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 3.2: Screening plates: Binding and wash/elute conditions

Screening plate	Binding conditions (µL/well)		Wash/elute conditions (µL/well)	
	2 or 6	20 or 50	2 or 6	20 or 50
AIEX screening or Capto AIEX polishing screening (see *Table 3.3* for details)	++	-	-	++
CIEX screening or Capto CIEX polishing screening (see *Table 3.4* for details)	++	-	-	++
HIC screening (see *Table 3.5* for details)	NA	++	-	++

Table 3.3: Resin distribution on AIEX screening and Capto AIEX polishing screening plates (2 µL/6 µL or 20 µL resin/well).

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	AIEX screenings plates: Capto Q (2 µL or 20 µL)			AIEX screenings plates: Capto DEAE (2 µL or 20 µL)			AIEX screenings plates: Q Sepharose Fast Flow (6 µL or 20 µL)			AIEX screenings plates: Capto adhere (6 µL or 20 µL)		
B												
C												
D	Capto AIEX polishing screening plates: Capto Q (2 µL or 20 µL)			Capto AIEX polishing screening plates: Capto Q ImpRes (6 µL or 20 µL)			Capto AIEX polishing screening plates: Capto adhere (6 µL or 20 µL)			Capto AIEX polishing screening plates: Capto adhere ImpRes (6 µL or 20 µL)		
E												
F												
G												
H												

Table 3.4: Resin distribution on CIEX screening and Capto CIEX polishing screening plates (2 µL/6 µL or 20 µL resin/well).

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	CIEX screenings plates: Capto S (2 µL or 20 µL)				CIEX screenings plates: SP Sepharose Fast Flow (6 µL or 20 µL)				CIEX screenings plates: Capto MMC (6 µL or 20 µL)			
B												
C												
D	Capto CIEX polishing screening plates: Capto S ImpAct (2 µL or 20 µL)				Capto CIEX polishing screening plates: Capto SP ImpRes (6 µL or 20 µL)				Capto CIEX polishing screening plates: Capto MMC ImpRes (6 µL or 20 µL)			
E												
F												
G												
H												

Table 3.5: Resin distribution on Capto HIC screening plate (50 µL resin/well).

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	Capto Phenyl High Sub			Capto Phenyl ImpRes			Capto Butyl		Capto Butyl ImpRes		Capto Octyl	
B												
C												
D												
E												
F												
G												
H												

Table 3.6: Distribution of resin volumes on isotherm plates (numbers in µL).

Well	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

4 Characteristics

PreDictor plates are disposable 96-well filter plates, each well prefilled with a defined amount of chromatography resin. The available resins are anion exchangers, cation exchangers, multimodal, hydrophobic interaction (HIC), and affinity resins, see *Table 1.1*. A barcode facilitates the identification of individual plates. *Table 4.1* and *Table 4.2* present available resins and characteristics of PreDictor plates, respectively.

Table 4.1: Characteristics of chromatography resins available in PreDictor plates.

Chromatography resin [1]	Characteristics	Matrix
Capto Q	Strong anion exchanger with high capacity	Highly cross-linked agarose with dextran surface extender
Capto S ImpAct	Strong cation exchanger with high capacity and high resolution	Highly cross-linked agarose
Capto Q ImpRes	Strong anion exchanger with high resolution	Highly cross-linked agarose
Capto SP ImpRes	Strong cation exchanger with high resolution	Highly cross-linked agarose
Capto DEAE	Weak anion exchanger with high capacity	Highly cross-linked agarose with dextran surface extender
Capto MMC	Multimodal weak cation exchanger	Highly cross-linked agarose
Capto MMC ImpRes	Multimodal weak cation exchanger with high resolution	Highly cross-linked agarose
Capto adhere	Multimodal strong anion exchanger	Highly cross-linked agarose
Capto adhere ImpRes	Multimodal strong anion exchanger with high resolution	Highly 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 SuRe LX	Alkali-stabilized protein A-derived ligand (*E. coli*). Designed for high titer cultures of monoclonal antibodies.	Highly cross-linked agarose
MabSelect PrismA	Protein A-derived ligand (E. coli) with enhanced alkali-stability. Exceptional productivity due to high binding capacity and high flow base matrix.	Rigid, highly cross-linked agarose
Capto L	Recombinant protein L (*E. coli*)	Highly cross-linked agarose
Capto Butyl	HIC resin	Highly cross-linked agarose

Chromatography resin [1]	Characteristics	Matrix
Capto Octyl	HIC resin	Highly cross-linked agarose
Capto Phenyl (high sub)	HIC resin	Highly cross-linked agarose

1 Details of the different chromatography resins are found in Data Files for respective resin, see *Chapter 8 Ordering information, on page 32*.

Table 4.2: PreDictor plate characteristics

Plate size	127.8 × 85.5 × 30.6 mm (according to ANSI/SBS 1-2004, 3-2004 and 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, 2]
Volume sedimented resin/well	2 µL, 6 µL, 20 µL or 50 µL For PreDictor isotherm plates different in different wells: 2 µL, 4 µL, 6 µL, 8 µL, 20 µL and 50 µL
Resin suspensions in total volume of	200 µL for 2 µL sedimented resin/well 500 µL for 6 µL, 20 µL, and 50 µL sedimented resin/well For PreDictor isotherm plates: 500 µL for 50 µL sedimented resin/well 200 µL for 20 µL sedimented resin/well 500 µL for 8 µL sedimented resin/well 375 µL for 6 µL sedimented resin/well 250 µL for 4 µL sedimented resin/well 125 µL for 2 µL sedimented resin/well
Storage solution	20% ethanol, 0.2 M sodium acetate for the following resins: PreDictor Capto MMC ImpRes Capto S ImpAct Capto SP ImpRes Capto CIEX polishing screening CIEX screening Capto SP ImpRes isotherm 20% ethanol for all other PreDictor plates
Recommended storage temperature	2°C to 8°C for all PreDictor plates
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 resin 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.

2 With working volume/well more than 300 µL for studies requiring longer incubation times, e.g., life time studies, the bottom of the filter plate should be sealed to avoid leakage from the plate.

5 Advice on handling

Equipment

PreDictor plates are designed for both manual and robotic handling. *Table 5.1* 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 5.1: Recommended equipment for manual and robotic handling of PreDictor plates

Equipment	Details	Tips and tricks
Pipette	Use an 8 or 12 multichannel 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 resin 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 micro-plate carriers capable of handling a PreDictor plate on top of a collection plate (for PreDictor plate size, see *Table 4.2*).	Centrifuge within 300 to 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 size, see *Table 4.2*).	The distance between the bottom of the PreDictor 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 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, 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.

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 lysis of the sample.

Before starting screening for HIC conditions, establish the “salt window” for the sample. Add increasing amount of salt to the sample to establish the concentration at which the precipitation occurs. Make sure that the sample is below this salt concentration before starting the screening experiments. Adjust the sample to the salt concentration of the binding buffers to promote hydrophobic interaction.

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 resin volume, see *Table 3.1*.
- In cases of persistent leakage, consider using a different detergent.

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. 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 *Assist software, on page 20*.

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 *Chapter 8 Ordering information, on page 32*.

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 resin particles are in contact with the sample, refer to handbook, High-throughput process development with PreDictor plates, for details.

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 5.1.

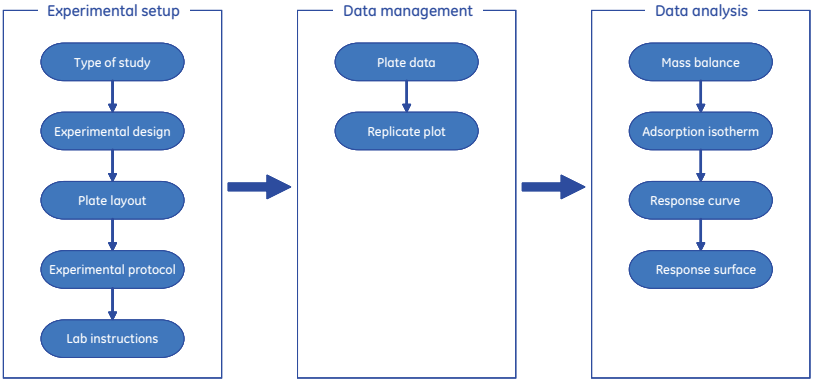


Figure 5.1: Assist software support to the HTPD workflow using PreDictor plates.

Experimental setup

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.

6 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 resin volume in wells. The PreDictor plates can be operated manually by using a multichannel 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*

General considerations

Storage

Upon storage, some of the storage solution may diffuse through the bottom filter of the PreDictor plates. This is seen as droplets on the bottom foil when the foil is removed. This diffusion is a very slow process that is likely caused by the relatively low surface tension of the ethanol solution. As soon as the plate has been rinsed with a full aqueous phase, e.g. water or equilibration buffer with higher surface tension, the membrane prohibits liquid transport and plates can be used with confidence that no leakage will occur during normal experimentation.

Automated operation

The *Detailed protocol, on page 23* 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 plate

It is important to carefully follow instructions for steps 1 and 2 in the protocol, see *Detailed protocol, on page 23*. If not followed there is a risk that chromatography resin 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 *Related products, on page 34*, or on another 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 (see *Chapter 8 Ordering information, on page 32*) or another appropriate spacer before further operation.
- Make sure 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 (see *Related products, on page 34*) 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 covering the PreDictor plate using a self-adhesive microplate foil (see *Related products, on page 34*) or another appropriate 96-well cover.

Mixing

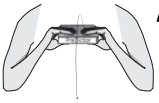
The PreDictor plate and the collection plate must be fixed to each other and to the microplate shaker during mixing. If the PreDictor plate outlets (the drips) rub against the edges of the collection plate wells, leakage may occur. For example, use a rubber band to secure the plates to each other and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

Sample and solution addition to PreDictor plates

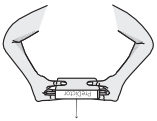
To minimize loading generated artefacts, add samples, buffers and solutions to the whole PreDictor plate without delay.

Detailed protocol

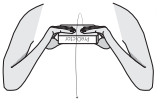
1 Resuspend the resin (20x)
To resuspend resin particles attached to the top seal, shake PreDictor plates as described (step 1A to 1D).



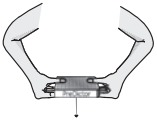
A Hold the PreDictor plate (top side up) with both hands. Keep the thumbs on the bottom side of the PreDictor plate and the other fingers on the top side.
Rotate the PreDictor plate to bottom side up while thrusting it downwards in a swift, controlled movement until the arms are fully extended.



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, turning the top side up again.

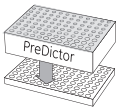


D Finish the movement with a flick downwards.

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 resin 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

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

or

Vacuum filtration, as described in step 3.

Perform the equilibration step at least three times or until the resin 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 resin volume, see Table 3.1.

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 a microplate foil (see *Related products, on page 34*) or an appropriate 96-well cover.

Note:

Incubation time is application related (see Section Related literature, on page 5 or Table Related literature, on page 35). 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

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

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

or

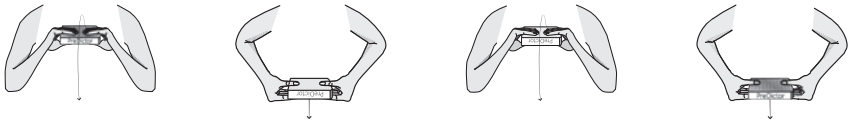
Vacuum filtration, as described in step 3.

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

Protocol quick guide

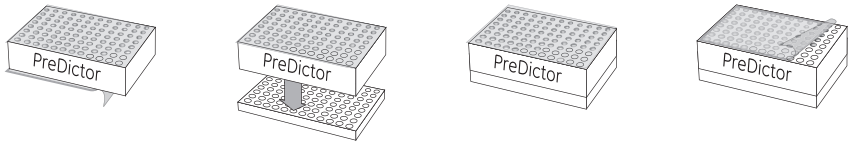
1 Resuspend the resin (20x: 10 times for each side)

A B C D



2 Remove cover seals

A B C D



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.

7 Troubleshooting guide

Problem	Possible cause	Corrective action
PreDictor plate wells are clogged.	The sample is too viscous.	Increase dilution of the cell paste before lysis, or dilute after the lysis.
	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. Make sure that the rubber gasket in the vacuum manifold tightens around the PreDictor plate. All wells should be emptied simultaneously.	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.	If the problem still occurs, change to centrifugation. When using centrifugation, different centrifugation forces may be tried (within the interval 300 to 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.
	The protein concentration is too high.	Reduce the protein concentration and/or use a PreDictor plate with another resin volume (see *Table 3.1*).

Problem	Possible cause	Corrective action
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.
	During a CIP (cleaning-in-place) study, the chromatographic resin may be fouled up to 10 times or more with crude sample.	Seal the bottom of the filter plate as soon as tendency for leakage occurs (see *Related products, on page 34*).
	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.

8 Ordering information

For information about related products, accessories, and related literature, refer to online information at: www.gelifesciences.com

PreDictor plates

Single resin plates	No. supplied	Product code no.
Ion exchange		
PreDictor Capto Q, 2 µL PreDictor Capto Q, 20 µL PreDictor Capto Q, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates 4 × 96-well filter plates	28925773 28925806 28925807
PreDictor Capto S ImpAct, 2 µL PreDictor Capto S ImpAct, 20 µL	4 × 96-well filter plates 4 × 96-well filter plates	17371716 17371717
PreDictor Capto Q ImpRes, 6 µL PreDictor Capto Q ImpRes, 20 µL	4 × 96-well filter plates 4 × 96-well filter plates	17547016 17547017
PreDictor Capto SP ImpRes, 6 µL PreDictor Capto SP ImpRes, 20 µL	4 × 96-well filter plates 4 × 96-well filter plates	17546816 17546817
PreDictor Capto DEAE, 2 µL PreDictor Capto DEAE, 20 µL PreDictor Capto DEAE, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates 4 × 96-well filter plates	28925811 28925812 28925813
Multimodal		
PreDictor Capto MMC, 6 µL PreDictor Capto MMC, 20 µL PreDictor Capto MMC, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates 4 × 96-well filter plates	28925814 28925815 28925816
PreDictor MMC ImpRes, 6 µL PreDictor MMC ImpRes, 20 µL	4 × 96-well filter plates 4 × 96-well filter plates	17371630 17371631
PreDictor Capto adhere, 6 µL PreDictor Capto adhere, 20 µL PreDictor Capto adhere, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates 4 × 96-well filter plates	28925817 28925818 28925819
PreDictor Capto adhere ImpRes, 6 µL PreDictor Capto adhere ImpRes, 20 µL	4 × 96-well filter plates 4 × 96-well filter plates	17371530 17371531
Affinity		
PreDictor MabSelect SuRe, 6 µL PreDictor MabSelect SuRe, 20 µL PreDictor MabSelect SuRe, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates 4 × 96-well filter plates	28925823 28925824 28925825
PreDictor MabSelect SuRe LX, 6 µL PreDictor MabSelect SuRe LX, 20 µL PreDictor MabSelect SuRe LX, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates 4 × 96-well filter plates	17547430 17547431 17547432

Single resin plates	No. supplied	Product code no.
PreDictor MabSelect PrismA, 6 µL PreDictor MabSelect PrismA, 20 µL PreDictor MabSelect PrismA, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates 4 × 96-well filter plates	17549830 17549831 17549832
PreDictor Capto L, 6 µL PreDictor Capto L, 20 µL PreDictor Capto L, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates 4 × 96-well filter plates	17547830 17547831 17547832
Hydrophobic Interaction		
PreDictor Capto Butyl, 6 µL PreDictor Capto Butyl, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates	17545916 17545917
PreDictor Capto Octyl, 6 µL PreDictor Capto Octyl, 50 µL	4 × 96-well filter plates 4 × 96-well filter plates	17546516 17546517

Screening plates	No. supplied	Product code no.
PreDictor Capto CIEX polishing screening (2 µL/6 µL) PreDictor Capto CIEX polishing screening (20 µL)	4 × 96-well filter plates 4 × 96-well filter plates	29095568 29095567
PreDictor Capto AIEX polishing screening (2 µL/6 µL) PreDictor Capto AIEX polishing screening (20 µL)	4 × 96-well filter plates 4 × 96-well filter plates	29095570 29095569
PreDictor AIEX screening (2 µL/6 µL) PreDictor AIEX screening (20 µL)	4 × 96-well filter plates 4 × 96-well filter plates	28943288 28943289
PreDictor CIEX screening (2 µL/6 µL) PreDictor CIEX screening (20 µL)	4 × 96-well filter plates 4 × 96-well filter plates	28943290 28943291
PreDictor Capto HIC screening, 50 µL	1 × 96-well filter plates	29305795

Adsorption isotherm plates	No. supplied	Product code no.
PreDictor Capto SP ImpRes isotherm	4 × 96-well filter plates	17546818 [1]
PreDictor Capto Q isotherm	4 × 96-well filter plates	28943278[1]
PreDictor Capto DEAE isotherm	4 × 96-well filter plates	28943280[1]
PreDictor Capto MMC isotherm	4 × 96-well filter plates	28943281[1]
PreDictor Capto adhere isotherm	4 × 96-well filter plates	28943282[1]
PreDictor MabSelect isotherm	4 × 96-well filter plates	28943283[1]
PreDictor MabSelect SuRe isotherm	4 × 96-well filter plates	28943284[1]

1 Plates are manufactured on request.

Assist software

Assist software is free to download from GE webpage.

Related products

Accessories	No. supplied	Product 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	28975816
Sealing Foil	100 x self-adhesive, aluminium foils	18114354

Prepacked columns	No. supplied	Product code no.
HiScreen Capto S ImpAct	1 x 4.7 mL	17371747
HiScreen Capto Q	1 x 4.7 mL	28926978
HiScreen Capto Q ImpRes	1 x 4.7 mL	17547015
HiScreen Capto SP ImpRes	1 x 4.7 mL	17546815
HiScreen Capto DEAE	1 x 4.7 mL	28926982
HiScreen Capto MMC	1 x 4.7 mL	28926980
HiScreen Capto MMC ImpRes	1 x 4.7 mL	17371620
HiScreen Capto adhere	1 x 4.7 mL	28926981
HiScreen Capto adhere ImpRes	1 x 4.7 mL	17371520
HiScreen MabSelect SuRe	1 x 4.7 mL	28926977
HiScreen MabSelect SuRe LX	1 x 4.7 mL	17547415
HiScreen MabSelect Xtra	1 x 4.7 mL	28926976
HiScreen MabSelect PrismA	1 x 4.7 mL	17549815
HiScreen Capto L	1 x 4.7 mL	17547814
HiScreen Capto Phenyl (high sub)	1 x 4.7 mL	28992472
HiScreen Capto Butyl	1 x 4.7 mL	28992473
HiScreen Capto Phenyl ImpRes	1 x 4.7 mL	17548411
HiScreen Capto Butyl ImpRes	1 x 4.7 mL	17371910

Related literature

Literature	Product code no.
Handbook	
High-throughput process development with PreDictor plates	28940358
Data file	
PreDictor 96-well filter plates	28925839
Application notes	
Screening of loading conditions on Capto S using a new high-throughput format, PreDictor plates	28925840
High-throughput screening of elution pH for monoclonal antibodies on MabSelect SuRe using PreDictor plates	28927792
Adsorption equilibrium isotherm studies using a high-throughput method	28940362
High-throughput screening and column optimization of a monoclonal antibody capture step	28940347
High-throughput screening and process development for capture of recombinant pro-insulin from *E. coli*	28996622
High Throughput Screening of HIC media in PreDictor plates for capture of recombinant Green Fluorescent Protein from *E. coli*	28996449
High-throughput process development for design of cleaning-in-place protocols	28984564
Mini-poster	
High-throughput screening of elution conditions on Capto MMC using PreDictor plates	28927790
Data files	
PreDictor RoboColumn	28988634
Capto S ImpAct	29067018
Capto S, Capto Q, and Capto DEAE	11002576
Capto SP ImpRes, and Capto Q ImpRes	28983763
Capto MMC	11003545
Capto MMC ImpRes	29027336
Capto adhere	28907888
Capto adhere ImpRes	29027332
MabSelect SuRe	11001165
MabSelect SuRe LX	28987062
MabSelect Xtra	11001157
MabSelect PrismA	KA553200917DF

Literature	Product code no.
Capto L	29010008
Capto Phenyl (high sub), Capto Butyl	28955857
Instructions/protocols	
Capto S ImpAct	29092501
Capto S, Capto Q, and Capto DEAE	28407452
Capto SP ImpRes, Capto Q ImpRes	28977655
Capto MMC	11003505
Capto MMC ImpRes	29027184
Capto adhere	28906405
Capto adhere ImpRes	29027182
MabSelect SuRe	11002601
MabSelect SuRe LX	28976500
MabSelect PrismA	29262586
Capto L	29003349

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