GE Healthcare

Instructions 28-9258-34 AB High-throughput process development

PreDictor plates

PreDictor

Contents

1 Introduction

PreDictor™ plates are disposable 96-well filter plates prefilled with GE Healthcare BioProcess™ chromatography media. 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 operated manually using multi-channel pipettes. Removal of liquid can be made either by centrifugation or vacuum filtration. Each well in a PreDictor plate is prefilled with a defined amount of chromatography medium. For each chromatography medium, three different plates with different volumes of media are available. The choice of medium volume depends on the type of application, as different applications require different amounts of medium in the wells (see section 2.2).

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. Once this experimental subspace has been found, optimization and scale-up may be done on columns using ÄKTAdesign™ systems, as all chromatography media are available in prepacked columns and in bulk packs.

Table 1. Available PreDictor plate products

Product	**Chromatography medium volume per well***
PreDictor Capto™ Q	2 µl or 20 µl or 50 µl
PreDictor Capto S	2 µl or 20 µl or 50 µl
PreDictor Capto DEAE	2 µl or 20 µl or 50 µl
PreDictor Capto MMC	6 µl or 20 µl or 50 µl
PreDictor Capto adhere	6 µl or 20 µl or 50 µl
PreDictor MabSelect™	6 µl or 20 µl or 50 µl
PreDictor MabSelect SuRe™	6 µl or 20 µl or 50 µl

* Medium suspensions in total volumes of:
2 µl sedimented medium/well: 200 µl
6 µl, 20 µl, and 50 µl sedimented medium/well: 500 µl

2 Applications

PreDictor plates can be used to screen different parts of the chromatographic cycle, for example determination of binding, wash, and elution conditions. Quantitative analysis of very low concentrations of proteins and/or impurities may be limited by non-specific adsorption to the filter plate. Regardless of the application, the workflow includes equilibration, sample addition, incubation, wash, and elution - thus, the same steps as a chromatographic cycle on a column.

Examples of protocols are described in Application Notes 28-9258-40 (Screening of loading conditions on Capto S using a new high-throughput format, PreDictor plates), 28-9277-90 (High-throughput screening of elution conditions on Capto MMC using PreDictor plates), and 28-9277-92 (High-throughput screening of elution pH for monoclonal antibodies on MabSelect SuRe using PreDictor plates).

2.1 Batch uptake experiment occurring in the wells of PreDictor plates

In a typical adsorption process, both a mass transfer mechanism responsible for protein transport and ligand selectivity are independent of the mode of operation (i.e., are the same 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. Therefore, 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.

Figure 1 shows a batch uptake experiment occurring 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.

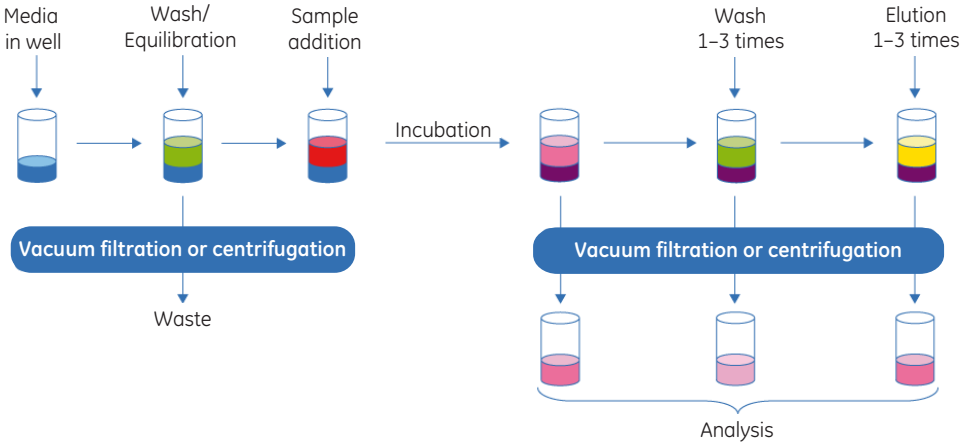


Fig 1. Schematic drawing of a batch uptake experiment occurring in the wells of PreDictor plates showing the same steps as in a chromatographic separation: equilibration, sample addition, wash and elution.

2.2 Different media volumes for different applications

For optimal results, different applications and samples will require different amounts of chromatography media in the wells. Thus, PreDictor plates with three different chromatography medium volumes (one volume per plate) are available to provide flexibility for designing a study. (For product overview, see Table 1).

When choosing PreDictor plate, the amount of material, target protein and impurities required for analysis need to be considered. 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. For guidance on plate selection, see Table 2.

2.2.1 Binding studies

In binding studies, relatively small volumes of chromatography medium are used. Overloading of the chromatography medium with protein is performed and the amount of unbound protein is measured. Alternatively, the amount of bound protein is determined after elution steps are performed. Generally, plates with 2 or 6 µl chromatography media should be used. Different volumes are used based on the properties of the media: for the high-capacity ion exchangers 2 µl is sufficient, while for the other media 6 µl is required for optimal results.

2.2.2 Wash and elution studies

For wash and elution studies, larger medium volumes may be required if sample purity needs to be determined. In such cases, minimum detectable amount of impurities will govern the choice of PreDictor plate. The first option for wash and elution studies is the 20 µl plate.

Table 2. PreDictor plate selection guide

Media volume in well (µl)	**Application binding conditions**					
	Capto Q	Capto S	Capto DEAE	Capto MMC	Capto adhere	MabSelect family
2	+ +	+ +	+ +	NA	NA	NA
6	NA	NA	NA	+ +	+ +	+ +
20	-	-	-	-	-	-
50	-	-	-	-	-	-

Media volume in well (µl)	**Application wash/elute conditions**					
	Capto Q	Capto S	Capto DEAE	Capto MMC	Capto adhere	MabSelect family
2	-	-	-	NA	NA	NA
6	NA	NA	NA	-	-	-
20	+ +[1]	+ +[1]	+ +[1]	+ +[1]	+ +[1]	+ +[1]
50	+[2]	+[2]	+[2]	+[2]	+[2]	+[2]

++ 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.

3 Properties of PreDictor plates and media

PreDictor plates are disposable 96-well filter plates made of polypropylene and polyethylene, each well with a total volume of 800 µl. Each well is prefilled with a defined amount of chromatography medium. For each of the chromatography media listed on page 5, three different plates with different volumes are available in order to fit different applications. To simplify the identification of individual plates, a barcode is placed on one of the short ends of the plate.

The available chromatography media are anion exchangers, cation exchangers, multimodal media, and affinity chromatography media for capture of monoclonal antibodies.

Tables 3 and 4 present characteristics of PreDictor plates and chromatography media, respectively.

Table 3. 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 *
Volume sedimented medium/well	Capto Q, Capto S, Capto DEAE: 2 µl, 20 µl, 50 µl Capto MMC, Capto adhere, MabSelect, MabSelect SuRe: 6 µl, 20 µl, 50 µl
Medium suspensions in total volume of	2 µl sedimented medium/well: 200 µl 6, 20, and 50 µl sedimented medium/well: 500 µl
Storage solution	All PreDictor plates except PreDictor Capto S: 20% ethanol PreDictor Capto S: 20% ethanol + 0.2 M sodium acetate
Recommended storage temperature	PreDictor Capto Q, PreDictor Capto S, PreDictor Capto DEAE, PreDictor Capto MMC and PreDictor Capto adhere: +4°C to +30°C PreDictor MabSelect and PreDictor MabSelect SuRe: +4°C to +8°C
Working temperature	+4°C to +30°C
Centrifugation force recommended maximum	Sample dependent 300 to 500 × *g* 700 × *g*

Vacuum recommended maximum	Sample dependent -0.15 to -0.3 bar -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 filter plate and containing: • Article number • Lot number • Individual identification number

* 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 filter plate.
Note: The volume and the amount of protein needed for analysis are also to be taken into consideration.

Table 4. 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
MabSelect	Recombinant protein A *(E. coli)*	Highly cross-linked agarose
MabSelect SuRe	Alkali-stabilized protein A-derived ligand *(E. coli)*	Highly cross-linked agarose

Details of the different chromatography media are found in data files: 11-0025-76 (Capto S, Capto Q, Capto ViralQ and Capto DEAE), 11-0035-45 (Capto MMC), 28-9078-88 (Capto adhere), 18-1149-94 (MabSelect), and 11-0011-65 (MabSelect SuRe).

4 Required equipment

Table 5 gives guidance over equipment required for manual and robotic handling of PreDictor plates. Tips and tricks provides guidance of working with PreDictor plate format.

Table 5. 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 filter plates.	When dispensing liquid it is useful to aspirate a larger volume and thereafter dispense the liquid into the filter plate wells in smaller fixed volumes in several steps. (Manual handling)
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 filter plate than the volume of the wells in the collection plate. When the collection plate is to be frozen, don't fill the wells to more than half of the handling volume. When using a UV readable collection plate, remember not to touch the bottom of the collection plate. (Manual handling)
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 media during incubation.	Avoid putting the filter plate directly on the lab bench or other surface. Always put the filter plate on a collection plate or other spacer to minimize risk of contamination and/or leakage. Safely secure the filter plate and the collection plate on the microplate shaker. For example, use a rubber band to secure the plates to each other. (Manual handling)
Centrifuge or	Use a swinging-bucket rotor with microplate carriers capable of handling a filter plate on top of a collection plate (for PreDictor plate size, see Table 3).	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.

Vacuum manifold	Designed and optimized for vacuum filtration of 96-well filter plates (for PreDictor plate size, see Table 3).	The distance between the bottom of the filter 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. First turn on the vacuum, then place the filter plate on the vacuum manifold. Set the vacuum within -0.15 to -0.5 bar. Turn off the vacuum when all solution is removed.
Reagent reservoir	Use a 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.	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 filter 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 filter plate, blot the bottom of the filter 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.

5 Experimental setup

By using Design of Experiments (DoE) for the experimental set-up, many different chromatographic conditions (factors) can be screened simultaneously in PreDictor plates. DoE employs statistics to identify and define the factors having the greatest impact on the process/product.

When using the high-throughput process development (HTPD) approach in PreDictor plates, it is recommended to screen a broader range of process parameters than usually done in columns, since this format provides this possibility in an efficient way. Examples of conditions to be screened may be:

- pH
- Conductivity/ionic strength
- Salt type
- Buffer species
- Additives (detergents, see section 8)

HTPD workflow may increase the amount of samples to analyze. One plate results in at least 96 samples to analyze. Consider suitable analytical methods, for example UV absorbance, ELISA, Biacore™ based assays (real time SPR), etc.

One product package containing four PreDictor plates is sufficient to perform for example 128 runs in a study when 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, page 26).

6 Sample preparation

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

7 How to use PreDictor plates

These protocols are 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 the filter plate.

7.1 General considerations

- The filter 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.
- To minimize the risk of contamination and/or leakage of sample and/or buffers through the filter in the filter plate, keep the filter plate on a collection plate (or an appropriate distance/spacer) throughout the whole workflow. Collection plates are not included and must be ordered separately. For example, use collection plates listed in Accessories, page 26.
- The filter plate and the collection plate must be fixed to each other and to the microplate shaker during mixing. If the filter 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.
- To minimize the risk of leakage of liquid through the filter in the plate, blot the bottom of the filter plate on a soft paper tissue. This removes drops of equilibration buffer that may have accumulated on the bottom of the filter plate. Blotting should always be done after centrifugation/vacuum filtration in the last equilibration step before sample loading. After blotting, the filter plate must be put on a collection plate before further operation.
- The filter plate may be covered by using a self-adhesive microplate foil (see Accessories, page 26) or an appropriate 96-well cover to avoid evaporation of liquid.

7.2 Protocol when using centrifugation

1 **Resuspending the medium**
In order to resuspend any medium particles attached to the top seal, PreDictor plates must be shaken in a controlled way. The resuspending procedure is illustrated in section 7.4 Protocol summary.

a. Hold the filter plate (top side up) with both hands. Keep the thumbs on the bottom side of the filter plate and the other fingers on the top side.
Rotate the filter 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 filter plate and the other fingers over (as above, but now with filter plate bottom up).
Repeat the rotation, making the top side up again.

d. Finish the movement with a flick downwards.

Repeat the rotations until the filter plate has been shaken 20 times (10 times for each side).

Note: It is strongly recommended to follow this guideline when opening the PreDictor plates, otherwise chromatography medium may remain attached to the top seal.

2 **Removal of cover seals**

a. Hold the filter plate horizontally and peel off the bottom seal.

b. Place the filter plate on a collection plate.

c. Let the filter plate rest for at least 1 minute to allow slurried medium to slide down from the filter plate walls.

d. Gently peel off the top seal from the filter plate while holding it against the collection plate.

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

3 **Removal of storage solution**
Centrifuge the plates for 1 minute at 500 × *g*, or until all storage solution is removed.

4 **Equilibration (3×)**
Add 200 µl equilibration buffer/well. Centrifuge for 1 minute at 500 × *g*. Perform the equilibration step at least three times or until the medium is equilibrated*.

5 **Blotting**
After centrifugation in the last equilibration step before sample loading, blot the bottom of the filter plate on a soft paper tissue to remove drops of equilibration buffer that may have accumulated on the bottom of the filter plate. After blotting, always place the filter plate on a collection plate before further operation.

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

6 **Sample loading**
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. For guidance on how to select the appropriate PreDictor plate, see Table 2.

Incubate on a microplate shaker at 1100 rpm. Fix the filter plate and the collection plate to each other and secure them to the microplate shaker during mixing. Incubation time is application related (see PreDictor plate literature, page 26). The top of the filter plate may be covered by using a microplate foil (see Accessories, page 26) or an appropriate 96-well

* Mixing may improve the efficiency of equilibration, wash and elution.

cover.
Remove supernatant by centrifugation for 1 minute at 500 × *g* or until all solution is removed.

Note: If covering the top of the filter plate, remove the cover before centrifugation.

7 **Wash out unbound sample (3×)**
Add 200 µl equilibration buffer/well. Centrifuge at 500 × *g* for 1 minute. Three wash steps are typically enough to remove all unbound sample*. Remember to change/empty the collection plate between each wash step.

Intermediate wash, if included in study (3×):
Add 200 µl wash buffer/well. Centrifuge at 500 × *g* for 1 minute. Three intermediate wash steps are typically enough to remove all unbound sample*. Remember to change/empty the collection plate between each intermediate wash step.

8 **Elution (3×)**
Add 200 µl of elution buffer/well as quickly as possible. Centrifuge at 500 × *g* for 1 minute. Three elution steps are typically enough to elute the sample*. Remember to change collection plates between each elution step.

7.3 Protocol when using vacuum

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

1 **Resuspending the medium**
In order to resuspend any medium particles attached to the top seal, PreDictor plates must be shaken in a controlled way. The resuspending procedure is illustrated in section 7.4 Protocol summary.

a. Hold the filter plate (top side up) with both hands. Keep the thumbs on the bottom side of the filter plate and the other fingers on the top side.
Rotate the filter 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 filter plate and the other fingers over (as above, but now with filter plate bottom up).
Repeat the rotation, making the top side up again.

d. Finish the movement with a flick downwards.

Repeat the rotations until the filter plate has been shaken 20 times (10 times for each side).

Note: It is strongly recommended to follow this guideline when opening the PreDictor plates, otherwise chromatography medium may remain attached to the top seal.

* Mixing may improve the efficiency of equilibration, wash and elution.

2 **Removal of cover seals**

a. Hold the filter plate horizontally and peel off the bottom seal.

b. Place the filter plate on a collection plate.

c. Let the filter plate rest for at least 1 minute to allow slurried medium to slide down from the filter plate walls.

d. Gently peel off the top seal from the filter plate while holding it against the collection plate.

3 **Removal of storage solution**
Set the vacuum pressure within -0.15 to -0.5 bar. Place the filter plate on the vacuum manifold, and vacuum filter until all storage solution is removed.

4 **Equilibration (3×)**
Add 200 µl equilibration buffer/well. Remove the buffer as in step 3 above. Perform the equilibration step at least three times or until the medium is equilibrated.*

5 **Blotting**
After vacuum filtration in the last equilibration step before sample loading, blot the bottom of the filter plate on a soft paper tissue to remove drops of equilibration buffer that may have accumulated on the bottom of the filter plate. After blotting, always place the filter plate on a collection plate before further operation.

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

6 **Sample loading**
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. For guidance on how to select the appropriate PreDictor plate, see Table 2.

Incubate on a microplate shaker at 1100 rpm. Fix the filter plate and the collection plate to each other and secure them to the microplate shaker during mixing. Incubation time is application related (see PreDictor plate literature, page 26). The top of the filter plate may be covered by using a microplate foil (see Accessories, page 26) or an appropriate 96-well cover.

Remove supernatant 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 filter plate on the vacuum manifold. Turn off the vacuum as soon as all solution is removed, to

* Mixing may improve the efficiency of equilibration, wash and elution.

avoid cross contamination in the collection plate.

Note: The distance between the bottom of the filter 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.

7 **Wash out of unbound sample (3×)**
Add 200 µl equilibration buffer/well. Remove the buffer as described in step 6 above. Three wash steps are typically enough to remove all unbound sample*. Remember to change/empty the collection plates between each wash step.

Intermediate wash, if included in study (3×):
Add 200 µl wash buffer/well. Remove the buffer as described in step 6 above. Three intermediate wash steps are typically enough to remove all unbound sample*. Remember to change/empty the collection plate between each intermediate wash step.

8 **Elution (3×)**
Add 200 µl of elution buffer/well as quickly as possible. Remove the eluate as described in step 6 above. Three elution steps are typically enough to elute the sample*. Remember to change collection plates between each elution step.

* Mixing may improve the efficiency of equilibration, wash and elution.

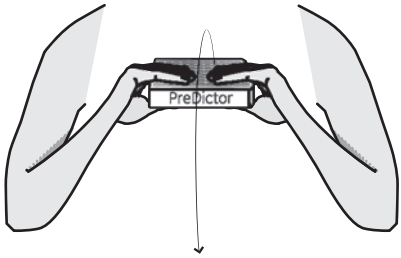
7.4 Protocol summary

This protocol is a summary of the general steps when working with PreDictor plates using centrifugation or vacuum.

1 Resuspending the medium

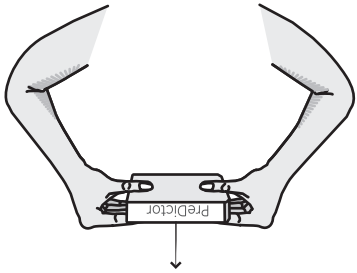
a. Hold the filter plate with both hands (top side up).
Rotate the filter plate to bottom side up while thrusting it downwards in a swift, controlled movement until the arms are fully extended.

a



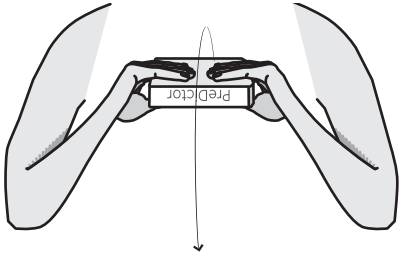
b. Finish the movement with a flick downwards.

b



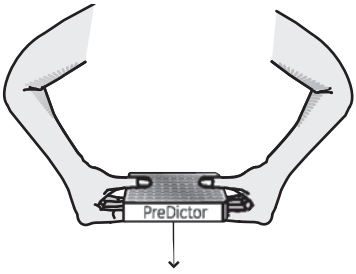
c. Reposition hands (filter plate bottom up). Repeat the rotation, making the top side up again.

c



d. Finish the movement with a flick downwards.

d



Repeat the rotations until the filter plate has been shaken 20 times (10 times for each side).

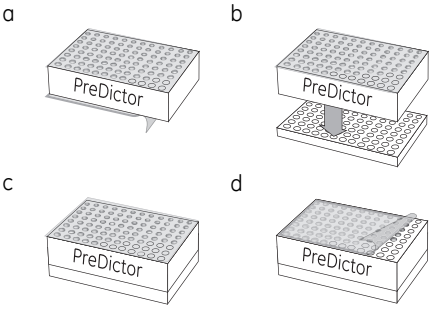
2 Removal of cover seals

a. Peel off the bottom seal.

b. Place the filter plate on a collection plate.

c. Let the filter plate rest for 1 min.

d. Gently peel off the top seal while holding the filter plate against the collection plate.



3 Removal of storage solution

- Centrifuge for 1 min at 500 × *g*.

  or

  Vacuum within -0.15 to -0.5 bar.

1 min
500 × *g*

-0.15 to -0.5 bar

4 Equilibration (3×)

- Add 200 µl equilibration buffer per well.
- Centrifuge for 1 min at 500 × *g*.

  or

  Vacuum within -0.15 to -0.5 bar.
- Perform the equilibration step at least 3 times.

Add 200 µl equilibration buffer.

1 min
500 × *g*

-0.15 to -0.5 bar

Perform 3 times.

5 Blotting

- Blot the bottom of the filter plate after the last equilibration step.
  Blotting may be added in other steps as well.

  Place the filter plate on a collection plate before further operation.

<table>
<tr><td colspan="3">6 Sample loading</td></tr>
<tr><td rowspan="3">• Add 100 to 300 µl sample per well.
• Incubate at 1100 rpm.
• Centrifuge for 1 min at 500 × g.
or
Vacuum within -0.15 to -0.5 bar.</td><td colspan="2">Add 100 to 300 µl sample.</td></tr>
<tr><td colspan="2">Incubate at 1100 rpm.</td></tr>
<tr><td> 
1 min
500 × g</td><td> 
-0.15 to -0.5 bar</td></tr>
<tr><td colspan="3">7 Washing (3×)</td></tr>
<tr><td rowspan="3">• Add 200 µl equilibration buffer per well.
• Centrifuge for 1 min at 500 × g.
or
Vacuum within -0.15 to -0.5 bar.
• Perform at least 3 times.</td><td colspan="2">Add 200 µl wash buffer.</td></tr>
<tr><td> 
1 min
500 × g</td><td> 
-0.15 to -0.5 bar</td></tr>
<tr><td colspan="2">Perform 3 times.</td></tr>
<tr><td colspan="3">8 Elution (3×)</td></tr>
<tr><td rowspan="3">• Add 200 µl elution buffer per well.
• Centrifuge for 1 min at 500 × g.
or
Vacuum within -0.15 to -0.5 bar.
• Perform at least 3 times.</td><td colspan="2">Add 200 µl elution buffer.</td></tr>
<tr><td> 
1 min
500 × g</td><td> 
-0.15 to -0.5 bar</td></tr>
<tr><td colspan="2">Perform 3 times.</td></tr>
</table>

8 Working with aqueous solutions containing detergents

PreDictor plates (the filter plates and the included chromatography media) 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 filter plate. The probability of leakage increases when using detergents with low surface tension. In general, the number of times the detergent is passing through the filter in the filter 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. For guidance of how to select the appropriate PreDictor plate, see Table 2 on page 8.
- In cases of persistent leakage, consider using a different detergent.

9 Troubleshooting guide

The following troubleshooting guide may be helpful when solving problems that may arise.

Fault	Possible cause	Action
Filter 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 filter plate and the collection plate is too large or too small.	• Reduce or increase the distance between the filter plate and the collection plate during vacuum filtration. The distance between the bottom of the filter 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 filter 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.
	• 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 filter plate during sample incubation	• The filter plate is not placed on a collection plate.	• During all handling of the filter 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 filter plate.	• Blot the bottom of the filter 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 step is important 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 filter plate, and is also time consuming.
	• The filter 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 filter 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 filter plate has been used in previous experiments.	• The filter plate is a disposable item. Always use new PreDictor plates when setting up new experiments.

10 Ordering information

For information about related products, accessories, and related literature, see online information. Unless otherwise stated, go to *www.gelifescience.com/bioprocess*. Enter the code number in the search function.

10.1 PreDictor plates

Product	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

10.2 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.
HiTrap™ Capto Q	5 × 1 ml	11-0013-02
HiTrap Capto S	5 × 1 ml	17-5441-22
HiTrap Capto DEAE	5 × 1 ml	28-9165-37
HiTrap Capto MMC	5 × 1 ml	11-0032-73
HiTrap Capto adhere	5 × 1 ml	28-4058-44
HiTrap MabSelect	5 × 1 ml	28-4082-53
HiTrap MabSelect SuRe	5 × 1 ml	11-0034-94

10.3 Related literature

PreDictor plate literature	Code no.
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
Application note: 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

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
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

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