HiTrap Capto Q ImpRes, 1 ml and 5 ml
HiTrap Capto SP ImpRes, 1 ml and 5 ml

Instructions for Use

Capto™ SP ImpRes and Capto Q ImpRes are strong cation and strong anion exchange BioProcess™ media, respectively, for intermediate purification and polishing of a wide range of biomolecules. The combination of the high flow agarose technique used for all Capto products and the small particle size of Capto ImpRes results in good pressure-flow properties as well as impressive resolution.

Capto SP ImpRes and Capto Q ImpRes provides:

- High resolution chromatography media on the well established Capto platform with traditional ligands
- Flexibility of design – large operational window of flow rates and bed heights
- Efficient design of robust polishing processes using convenient and scalable tools and formats
- High productivity enabling improved process economy

HiTrap™ Capto Q ImpRes and HiTrap Capto SP ImpRes are prepacked 1 ml and 5 ml columns for screening of selectivity, binding and elution conditions, as well as small scale purifications. HiTrap Capto Q ImpRes and Capto SP ImpRes columns provide fast, reproducible and easy separations in a convenient format. The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™

Table of contents

1. Product description 3
2. Performing a separation 6
3. Optimization 12
4. Cleaning 14
5. Adjusting pressure limits in chromatography system software ... 15
6. Storage 16
8. Ordering information 17

Important

Please read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns 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 product in a safe way, please refer to the Safety Data Sheet.

1 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

Fig 1. HiTrap, 1 ml column.

Fig 2. HiTrap, 5 ml column.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 ml	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 x 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/ 16"	For sealing bottom of HiTrap column	2, 5 or 7

Chromatography medium properties

The structures of the well established SP (sulfonate group) and Q (quartenary amine group) ligands used for Capto ImPres are shown in Figure 3.

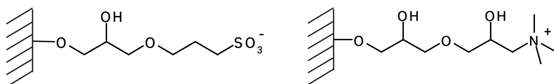

Fig 3. The strong ion exchange group of Capto SP ImpRes (left) and Capto Q ImpRes (right)

The media are designed for intermediate purification or polishing. They are based on the high flow agarose base matrix, which gives good pressure-flow properties.

The bead size is small (40 µm), which allows for high resolution. The combination of the well established SP and Q ligands with a small high flow agarose bead makes the chromatography media ideal for high throughput intermediate purification and polishing. Further characteristics of the two different media are found in Table 2.

Table 2. Characteristics of Capto SP ImpRes and Capto Q ImpRes

	Capto SP ImpRes	Capto Q ImpRes
Matrix	High flow agarose	High flow agarose
Functional group	$-CH_2CH_2CH_2SO_3^-$	$-CH_2N^+(CH_3)_3$
Total ionic capacity	0.13 to 0.16 mmol (H^+) /ml medium	0.15 to 0.18 mmol (Cl^-) /ml medium
Particle size (d_{50v})[1]	36 to 44 µm	36 to 44 µm
Flow velocity[2]	Typically ≈400 cm/h (<4 bar or 0.4 MPa) or ≈300 cm/h (<3 bar or 0.3 MPa) in a packed bed in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water.	
Binding capacity[3]	>70 mg lysozyme /ml medium >95 mg BSA /ml medium	>55 mg BSA /ml medium >48 mg β-lactoglobulin /ml medium
Operating pH stability[4]		
- short term	3 to 14	2 to 14
- long term	4 to 12	2 to 12
Working temperature	4°C to 30°C	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M sodium hydroxide[5], 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol	
Avoid	Oxidizing agents, cationic detergents.	Oxidizing agents, anionic detergents.
Storage	20% ethanol, 0.2 M sodium acetate	20% ethanol

[1] d_{50v} is the median particle size of the cumulative volume distribution.

[2] Minimum values are >300 cm/h (<4 bar or 0.4 MPa) or >220 cm/h (<3 bar or 0.3 MPa) given the particle size specification 36 to 44 µm.

[3] Dynamic binding capacity at 10% breakthrough measured at a residence time of 4 minutes (150 cm/h) in a Tricorn™ 5/100 column with 10 cm bed height.

[4] Short term pH: pH interval where the medium can be subjected to for cleaning- or sanitization-in-place without significant change of function.
Long term pH: pH interval where the medium can be operated without significant change of function.

[5] No significant change in ionic capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

2 Performing a separation

Sample preparation

Adjust the sample to the composition of the start buffer by buffer exchange using for example HiTrap Desalting or HiPrep™ 26/10 Desalting columns, see . Before application to the column, the samples can be centrifuged or filtered through a 0.45 µm filter.

Sample preparation without clarification of sample

The following sample preparation procedure is aimed to give a sample, sufficiently homogenized to be applied directly to the column without prior clarification. The protocol below has been used successfully in our own laboratories, but other established procedures may also work.

1. Dilution of cell paste: Add 5–10 ml of start buffer for each gram of cell paste.
2. **Enzymatic lysis:** 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM Pefabloc™ SC or PMSF (final concentrations). Stir for 30 min at room temperature or 4°C depending on the sensitivity of the target protein.

3 **Mechanical lysis:** Sonication on ice for approx. 10 min, homogenization with a French press or other homogenizer or freeze/thaw, repeated at least five times.

Mechanical lysis time may have to be extended compared with standard protocols to secure an optimized lysate for sample loading (to prevent clogging of the column and back pressure problems). Different proteins have different sensitivity to cell lysis and care must be taken to avoid frothing and overheating of the sample.

4 Adjust the pH of the lysate. The pH should be at least 0.5 units below (cation exchangers) or 0.5 units above (anion exchangers) the pI of the target molecule. Do not use strong bases or acids for pH-adjustment (precipitation risk). Apply the unclarified lysate on the column directly after preparation.

Note: *If the sonicated or homogenized unclarified cell lysate is frozen before use, precipitation and aggregation may increase. New sonication of the lysate can then prevent increased back pressure problems when loading on the column.*

Table 3. Prepacked columns for desalting and buffer exchange

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting [1]	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting [2]	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting [3]	1.0 to 2.5 mL[4] 1.75 to 2.5 mL[5]	3.5 mL Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL[4] 0.2 to 0.5 mL[5]	1.0 mL Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL[4] 0.75 to 1 mL[5]	1.5 mL Up to 1 mL

[1] Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

[2] Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

[3] Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

[4] Volumes with gravity elution.

[5] Volumes with centrifugation.

Choice of start and elution buffer

The elution buffer is usually of the same composition and pH as the start buffer, but it contains additional salt, most often sodium chloride. The pH of the start buffer should be at least 0.5–1 pH unit above the pI of the target molecule when using an anion exchanger and at least 0.5-1 pH unit below the pI when using a cation exchanger.

The buffer species and buffer concentration are important for reproducible and robust methods. Table 4 and Table 5 show suitable buffers for anion and cation exchangers, respectively, and suggested starting concentrations. The buffer concentration should be at least 10 mM, and only rarely above 100 mM.

For samples with unknown charge properties, try the following:

Anion exchange (Q)

Start buffer: 20 mM Tris-HCl, pH 8.0

Elution buffer: 20 mM Tris-HCl, 1 M NaCl, pH 8.0

Cation exchange (SP)

Start buffer: 50 mM sodium acetate, pH 5.0

Elution buffer: 50 mM sodium acetate, 1 M NaCl, pH 5.0

or

Start buffer: 50 mM MES, pH 6.0

Elution buffer: 50 mM MES, 1 M NaCl, pH 6.0

Table 4. Buffers for anion exchange chromatography.

pH interval	Substance	Conc. (mM)	Counter-ion	pK_a (25°C)[1]
4.3–5.3	N-Methylpiperazine	20	Cl^-	4.75
4.8–5.8	Piperazine	20	Cl^- or $HCOO^-$	5.33
5.5–6.5	L-Histidine	20	Cl^-	6.04
6.0–7.0	Bis-Tris	20	Cl^-	6.48
6.2–7.2	Bis-Tris propane	20	Cl^-	6.65
8.6–9.6	Bis-Tris propane	20	Cl^-	9.10
7.3–8.3	Triethanolamine	20	Cl^- or CH_3COO^-	7.76
7.6–8.6	Tris	20	Cl^-	8.07
8.0–9.0	N-Methyldiethanolamine	20	SO_4^{2-}	8.52
8.0–9.0	N-Methyldiethanolamine	50	Cl^- or CH_3COO^-	8.52
8.4–9.4	Diethanolamine	20 at pH 8.4 50 at pH 8.8	Cl^-	8.88
8.4–9.4	Propane 1,3-diamino	20	Cl^-	8.88
9.0–10.0	Ethanolamine	20	Cl^-	9.50
9.2–10.2	Piperazine	20	Cl^-	9.73
10.0–11.0	Propane 1,3-diamino	20	Cl^-	10.55
10.6–11.6	Piperidine	20	Cl^-	11.12

[1] Handbook of chemistry and physics, 83rd edition, CRC, 2002–2003.

Table 5. Buffers for cation exchange chromatography

pH interval	Substance	Conc. (mM)	Counter-ion	pK_a (25°C)[1]
1.4–2.4	Maleic acid	20	Na^+	1.92
2.6–3.6	Methyl malonic acid	20	Na^+ or Li^+	3.07
2.6–3.6	Citric acid	20	Na^+	3.13
3.3–4.3	Lactic acid	50	Na^+	3.86
3.3–4.3	Formic acid	50	Na^+ or Li^+	3.75
3.7–4.7	Succinic acid	50	Na^+	4.21
5.1–6.1	Succinic acid	50	Na^+	5.64
4.3–5.3	Acetic acid	50	Na^+ or Li^+	4.75
5.2–6.2	Methyl malonic acid	50	Na^+ or Li^+	5.76
5.6–6.6	MES	50	Na^+ or Li^+	6.27
6.7–7.7	Phosphate	50	Na^+	7.20
7.0–8.0	HEPES	50	Na^+ or Li^+	7.56
7.8–8.8	BICINE	50	Na^+	8.33

[1] Handbook of chemistry and physics, 83rd edition, CRC, 2002-2003.

First time use or after long term storage

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml).

1. Remove the stopper and connect the column to the system (or syringe) with a drop-to-drop connection to avoid introducing air into the column.
2. Remove the snap-off end at the column outlet and wash with 1 column volume (CV) of distilled water. This step ensures removal of ethanol and avoids the precipitation of buffer salts upon exposure to ethanol. The step can be omitted if precipitation is not likely to be a problem.
3. Wash with 5 CV of start buffer.
4. Wash with 5 CV of elution buffer.
5. Wash with 5 CV of start buffer.

Separation by gradient elution

Linear ionic strength gradients should always be used for method development or when starting with an unknown sample. Linear ionic strength gradients are easy to prepare and very reproducible when generated by a suitable chromatography system. The results obtained can then serve as a base from which to optimize the separation.

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml). Collect fractions throughout the separation.

1. Equilibrate the column with at least 5 column volumes (CV) of start buffer for Capto Q ImpRes and Capto SP ImpRes or until the UV baseline, eluent pH and conductivity are stable.
2. Adjust the sample to the chosen starting pH and conductivity and apply to the column.
3. Wash with 5–10 CV of start buffer or until no material appears in the effluent.
4. Begin elution using a gradient volume of 10–20 CV and an increasing salt concentration up to 0.5 M NaCl (50% elution buffer).
5. Wash with 5 CV of 1 M NaCl (100% elution buffer) to elute any remaining ionically bound material.
6. Re-equilibrate with 5–10 CV of start buffer or until the UV baseline, eluent pH, and conductivity reach the required values.

Screening of selectivity

HiTrap columns are a convenient format for screening the selectivity of different ion exchange chromatography media.

3 Optimization

Screening for optimal loading conditions

Scout for optimal loading conditions by testing a range of pH values within which the target protein is known to be stable. If the isoelectric point of the target protein is known, then begin with a narrower pH range, for example, 0.5–1 pH unit away from the isoelectric point. In some cases the sample conductivity is equally important as the pH when scouting for optimal loading conditions. We therefore also recommend to scout for optimal ionic strength by varying the conductivity of the sample between 2–15 mS/cm.

Users of ÄKTA design systems with BufferPrep or BufferPro functionality can select from a range of buffer recipes to test media over a range of pH values and elution conditions.

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml). Collect fractions throughout the separation.

1. Decide what pH values and conductivities are to be investigated. Prepare samples according to this.
2. Start buffers: set up a series of buffers with pH values in the range 5–9 (Capto Q ImpRes) or 4–8 (Capto SP ImpRes) and with 0.5–1 pH unit intervals between each buffer. See Table 4 and Table 5 for recommended buffers. Where the conductivity of the buffers should be considered, it can either be adjusted by increasing the buffer concentration or adding sodium chloride.
3. Elution buffers: set up a second series of buffers with the same pH values, but including 1 M NaCl.
4. Equilibrate with at least 5 column volumes (CV) of start buffer for Capto Q ImpRes and Capto SP ImpRes or until the UV baseline, eluent pH and conductivity are stable.
5. Apply a known amount of the sample.
6. Wash with at least 5 CV of start buffer or until no material appears in the effluent.
7. Elute bound material with elution buffer (3–5 CV is usually sufficient, but other volumes may be required dependent on the exact experimental conditions).

8 Analyze all fractions (for example by an activity assay) and determine purity and the amount bound to the column.
9 Perform steps 4–8 for the next buffer pH.
10 Select pH and conductivity: the most suitable buffer should a) allow the target protein to bind and b) recover the protein with as high purity as possible.

Separation by step elution

Reduce separation time and buffer consumption by transferring to a step elution.

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml). Collect fractions throughout the separation.

1. Equilibrate the column with at least 5 column volumes (CV) of start buffer for Capto Q ImpRes and Capto SP ImpRes or until the UV baseline, eluent pH, and conductivity are stable.
2. Adjust the sample to the chosen starting pH and conductivity and apply to the column.
3. Wash with 5–10 CV of start buffer or until no material appears in the effluent.
4. Elute with 5 CV of start buffer including NaCl at chosen concentration.
5. Repeat step 4 at higher NaCl concentrations until the target protein has been eluted.
6. Wash with 5 CV of a high salt solution (1 M NaCl in start buffer) to elute any remaining ionically bound material.
7. Re-equilibrate with 5–10 CV of start buffer or until the UV baseline, eluent pH, and conductivity reach the required values.

Save time by using higher flow rates during the high salt wash and re-equilibration steps. Do not exceed the maximum recommended flow and back pressure for the column.

Further optimization

HiTrap columns are best suited for initial screening of binding and elution conditions, further optimization is preferably done on a larger column such as prepacked HiScreen™ (4.7 ml) and HiScale™ columns.

4 Cleaning

Correct preparation of samples and buffers, including a high salt wash (1–2 M NaCl) after each purification, should maintain columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the medium needs cleaning.

The following procedure removes common contaminants:

Flow: 1 ml/min (HiTrap 1 ml), 5 ml/min (HiTrap 5 ml).

1. Wash with at least 2 column volumes (CV) of 2 M NaCl.
2. Wash with at least 4 CV of 1 M NaOH.
3. Wash with at least 2 CV of 2 M NaCl.
4. Rinse with at least 2 CV of distilled water.
5. Wash with 5 CV of start buffer for Capto Q ImpRes and Capto SP ImpRes or until eluent pH and conductivity have reached the required values.

Note: *For some contaminants a more rigorous CIP procedure can be required. For more details, see instructions "Capto SP ImpRes and Capto Q ImpRes", code number 28-9776-55.*

5 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure 4. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictorExceeding the flow limit (see Table 2) may damage the column.

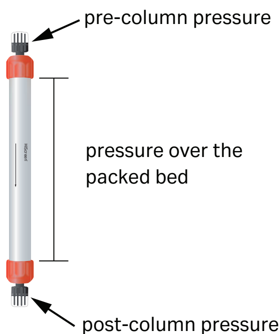

Fig 4. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured

value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

1. Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2. Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3. Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

   The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

6 Storage

Wash with 2 column volumes (CV) of distilled water followed by 2 CV of 20% ethanol (Capto Q ImpRes) or 20% ethanol containing 0.2 M sodium acetate (Capto SP ImpRes).
Store at 4°C to 30°C. Do not freeze.
Ensure that the column is sealed well to avoid drying out.

7 Further information

Further general information about optimization, troubleshooting, cleaning, and other topics can be found in the Handbook, *Ion Exchange Chromatography & Chromatofocusing, Principles and Methods*, *see* "Ordering information"

8 Ordering information

Product	Quantity	Code No.
HiTrap Capto Q ImpRes	5 × 1 ml	17-5470-51
	5 × 5 ml	17-5470-55
HiTrap Capto SP ImpRes	5 × 1 ml	17-5468-51
	5 × 5 ml	17-5468-55

Related Products	Quantity	Code No.
Capto Q ImpRes	25 ml	17-5470-10
	100 ml	17-5470-02
	1 L[1]	17-5470-03
Capto SP ImpRes	25 ml	17-5468-10
	100 ml	17-5468-02
	1 L[1]	17-5468-03
HiScreen Capto Q ImpRes	1 × 4.7 ml	17-5470-15
HiScreen Capto SP ImpRes	1 × 4.7 ml	17-5468-15
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

[1]Process scale quantities are available. Please contact your local representative.

Related literature	Code No.
Handbook: Ion Exchange Chromatography & Chromatofocusing, Principles and Methods	11-0004-21
Ion Exchange Chromatography Columns and Media, Selection Guide	18-1127-31
Prepacked chromatography columns for ÄKTA design systems, Selection Guide	28-9317-78

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16” female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16” male *(For connection to original FPLC System through top of HiTrap column*)	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16” *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Page intentionally left blank

cytiva.com/hitrap

Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate.

ÄKTA, BioProcess, Capto, HiPrep, HiScale, HiScreen, HiTrap, MidiTrap, MiniTrap, Sephadex and Tricorn are trademarks of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva.

Pefabloc is a trademark of DSM IP Assets B.V.

All other third party trademarks are the property of their respective owners.

© 2020 Cytiva

All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.

For local office contact information, visit cytiva.com/contact.

28980302 AC 10/2020