StrepTrap XT, 1 mL and 5 mL

Prepacked columns

Instructions for Use

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Abstract

StrepTrap™ XT is a ready to use HiTrap™ column prepacked with Strep-Tactin™ XT Sepharose™, a resin for purifying recombinant proteins with Strep-tag™ II or Twin-Strep-tag.
Purification is done under physiological conditions and mild elution preserves the activity of the target protein. Thanks to the high specificity of the binding, very high purity is achieved in just one step.
The design of the HiTrap column, together with the robust, high-resolution prepacked resin provides fast, simple and easy separations in a convenient format.

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Important

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 product in a safe way, 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. StrepTrap XT columns can be operated with a syringe, a laboratory pump or a liquid chromatography system such as ÄKTA™.

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	0.5 MPa (5 bar)	0.5 MPa (5 bar)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography resin, 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

Resin properties

StrepTrap XT 1 mL and 5 mL columns are prepacked with Strep-Tactin XT Sepharose. This robust, high-resolution resin is based on the 34 µm Sepharose High Performance matrix. Due to the small size of the beads bound protein is eluted in a narrow peak minimizing the need for further concentration steps.

The immobilized ligand is a specially-engineered streptavidin enabling purification of recombinant proteins with Strep-tag II or Twin-Strep-tag. Purification is performed under physiological conditions and mild elution using biotin preserves the activity of the target protein. The mild conditions even allow purification of intact protein complexes. Strep-Tactin XT Sepharose is the further development of StrepTactin Sepharose HP, offering higher affinity and enables purification under denaturing conditions. Strep-Tactin XT Sepharose has a high binding affinity in the low pM range for Twin-Strep-Tag.

Table 2 summarizes the characteristics of prepacked StrepTrap XT columns.

Table 2. Characteristics of StrepTrap XT.

Matrix	Rigid highly cross-linked 6% agarose
Average particle size	34 µm
Ligand	Strep-Tactin XT
Ligand concentration	Approx. 5 mg/mL resin
Dynamic binding capacity[1]	Approx. 10 mg protein with Strep-tag II or Twin-Strep-tag/mL resin
Recommended flow rates	1 and 5 mL/min for 1 and 5 mL columns respectively
Maximum flow rates[2]	4 and 20 mL/min for 1 and 5 mL columns respectively
Chemical stability	Stable in all commonly used aqueous buffers (see Table 3)
pH working range	6 to 10
Storage	2°C to 8°C in 20% ethanol

1) Binding capacity is protein dependent. Dynamic binding capacity (DBC) is defined as mg protein applied per mL resin at the point where the concentration of protein in the column effluent reaches a value of 10% of the concentration in the sample.

DBC was tested here at a flow rate of 1 mL/min in a 1 mL HiTrap column (1 min residence time) for GAPDH-Twin-Strep-tag (M_r 39 400) and GAPDH-Strep-tag II (M_r 37 400) in 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.

2 H_2O at room temperature.

Strep-Tactin XT Sepharose is compatible with a wide range of additives (see Table 3)

Table 3. Compatibility of Strep-Tactin XT Sepharose with different additives[1]

Reagent	Concentration
NaCl	5 M
$MgCl_2$[2]	1 M
EDTA	50 mM
β-mercaptoethanol	45 mM
Guanidine hydrochloride[2]	4 M
Urea[2]	6 M
Tween™ 20	2%
SDS, Sodium-N-dodecyl sulphate[2]	0.09%
Glycerol	25%
Ethanol	10%
Imidazole	250 mM

Note: These reagents have been successfully tested for purifying e.g., mCherry-Twin-Strep-tag or GAPDH-Twin-Strep-tag, with concentrations up to those listed. Higher concentrations may, however, be possible. Since binding depends on the sterical accessibility of the affinity tag in the context of the particular protein, the possible concentration may deviate from the given value for other proteins.

Note: It is not recommended to include DTT in samples or buffers.

[1] Data kindly provided by IBA GmbH, Germany, the manufacturer and IP owner of the Strep-Tactin XT ligand.

[2] Purification with SDS, $MgCl_2$, urea or guanidine hydrochloride is possible. However, maximum protein binding capacity and recovery might be reduced by up to 50%.

2 General considerations

Strep-tag II is a small tag of only eight amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) and and a molecular weight of M_r =1000. The small size of the tag makes it very useful as it will generally not interfere with structural and functional studies. Thus, it is not always necessary to cleave it off.

Twin-Strep-tag is a sequential arrangement of two Strep-tag II sequences with increased affinity for Strep-Tactin XT Sepharose.

Purification is done under physiological conditions, which together with mild elution by biotin preserves the activity of the target protein.

Regeneration of the resin is recommended before performing the next purification run on the same column. This is fast and easy to perform using 50 mM NaOH.

3 Operation

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.

Recommended buffers

Binding buffer	100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8
Elution buffer	50 mM biotin in binding buffer
Regeneration buffer	50 mM NaOH

Preparation of elution buffer

500 mL elution buffer can be prepared as described below:

1 Dissolve 6.1 g biotin (M_r 244.31),
4.4 g NaCl (58.44 g/mol),
6.1 g tris(hydroxymethyl)amino methane (121.14 g/mol), and 0.2 g disodium EDTA dihydrate (372.24 g/mol) in 450 mL distilled water.

2 Adjust the pH to 8.0 using HCl and add distilled water to 500 mL.

Sample preparation

Adjust the sample to the composition of the binding buffer. Either dilute the sample with binding buffer or exchange buffer using a column for desalting, refer to Table 4.

To avoid clogging the column, clarify the sample by centrifugation and filtration through a 0.45 µm filter immediately before application.

Prepacked columns for desalting

Table 4. Columns prepacked with Sephadex™ G-25.

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 [3]	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 [3]	0.5 to 1 mL [4] 0.75 to 1 mL [5]	1.5 mL Up to 1 mL

1) Requires a chromatography system to run.

2) Requires a syringe or a chromatography system to run.

3) Can be run by gravity flow or centrifugation.

4) Volumes with gravity elution.

5) Volumes with centrifugation.

Purification

Note: *The recommended operating flow rate for StrepTrap XT is 1 and 5 mL/min for 1 and 5 mL columns respectively.*

3 Remove the stopper from the inlet and the snap-off end at the column outlet.

4 Connect the column to a syringe (use the luer connector provided), pump tubing or chromatography system tubing (use 1/16" male connectors (28401081)).

Note: *Make a drop-to-drop connection to prevent air from entering the column.*

Note: *Make sure that the connectors are tight to prevent leakage.*

5. Wash the column with 5 column volumes (CV) of distilled water to remove ethanol.
6. Equilibrate the column with at least 5 CV binding buffer.
7. Load the sample onto the column.
8. Wash with 5 to 10 CV binding buffer or until no material appears in the effluent.
9. Elute with 6 CV elution buffer.
10. If required, the eluted fractions can be buffer exchanged. Refer to Table 4 for recommended columns.

Regeneration

1. Regenerate the column with 3 CV distilled water followed by 3 CV 50 mM NaOH and 3 CV distilled water. Use a flow rate of 1.0 mL/min or 5.0 mL/min for 1 mL and 5 mL columns respectively.
2. Immediately re-equilibrate the column with 8 CV binding buffer.

Cleaning-in-place (CIP)

CIP removes very tightly bound, precipitated or denatured substances from the resin. The accumulated contaminants can affect the chromatographic properties of the prepacked column, reduce the capacity, or contaminate the subsequent runs. Use up to 0.5 M NaOH for cleaning the column. Use the same protocol as in *Regeneration.* Avoid incubating with NaOH for a longer period as this can permanently reduce the binding capacity.

6 M urea or 4 M GuHCl can also be used to remove non-specifically bound proteins.

If removal of DNA is necessary, use a high concentration of NaCl (up to 5 M).

4 Scaling up

Scaling up from 1 mL to 5 mL StrepTrap XT column is easily performed by increasing sample load and flow rate five-fold.

An alternative method for quick scale-up is to connect two or three StrepTrap XT columns in series (back pressure will increase).

Strep-Tactin XT Sepharose is also available in 10 and 50 mL lab packs.

5 Adjusting pressure limits in chromatography system software

The pressure generated by the flow through a column affects the packed bed and the column hardware, refer to Figure 3. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rate
- High viscosity for buffers or sample
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit can damage the column, refer to Table 2.*

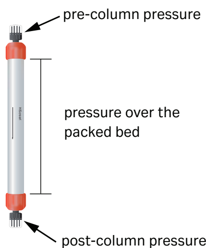

Fig 3. Precolumn and post-column pressure measurements.

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The system will automatically monitor the pressures (precolumn pressure and pressure over the packed bed, Δp). The precolumn pressure limit is the column hardware pressure limit refer to Table 1.

The maximum pressure the packed bed can withstand depends on resin 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 the optimal functionality in ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with pressure sensor in the pump, the pressure limit in the software can be adjusted as follows:

1

- Replace the column with a piece of tubing.
- Run the pump at the maximum intended flow rate.
- Record 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.
- Record 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.

Result: The actual pressure over the packed bed (Δp) during the run is equal to the actual measured pressure minus the total system pressure (P1).

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

6 Storage

Store StrepTrap XT columns in 20% ethanol at 2°C to 8°C.

After storage, equilibrate with binding buffer before use.

7 Troubleshooting

The following tips may be of assistance. If you have further questions about your StrepTrap XT column, please visit *cytiva.com/hitrap* or contact our technical support or your local Cytiva representative.

High back pressure:

- Increase the efficiency of the mechanical cell disruption e.g. increase sonication time. (Keep the sample on ice during sonication to avoid frothing and overheating as this may denature the target protein. Over-sonication can also lead to co-purification of host proteins with the target protein).
- Increase dilution of the cell paste before mechanical lysis, or dilute after lysis to reduce viscosity.
- If the lysate is very viscous due to a high concentration of host nucleic acid, continue sonication until the viscosity is reduced, and/or add additional DNAse. Alternatively, draw the lysate through a syringe needle several times.
- Freezing/thawing the lysate may increase precipitation and aggregation. Sonicating the thawed lysate can prevent increased backpressure problems when loading on the column. Clarify the sample by centrifugation and filtration through a 0.45 μm filter.
- If the purification has been performed at 4°C, try repeating it at room temperature if possible (sample viscosity is reduced at room temperature).
- Decrease flow rate during sample loading.

Column has clogged:

- Clean the column as described in Sub section “Cleaning-in-place (CIP)” on page 10.
- Replace the column. Optimize sample pretreatment before loading the next sample.

No or weak binding to StepTrap XT column:

- Protein has precipitated on the column: Clean the column according to instructions under Operation. In the following run, decrease the amount of sample or decrease protein concentration by eluting with a linear gradient instead of stepwise elution. Try detergents or change the NaCl concentration.
- Protein found in the flowthrough: Buffer/sample composition is not optimal; check the pH and composition of the sample and binding buffer. pH should be above pH 6.
- Column capacity is exceeded*:* If a StrepTrap XT 1 mL column has been used, change to the larger StrepTrap XT 5 mL. For quick scale-up, connect two or more columns in series. Note that connecting columns in series will increase backpressure.
- Strep-tag II or Twin-Strep-tag are not present:
  Use protease-deficient *E. coli* expression strains. Add protease inhibitors during cell lysis.
- Strep-tag II or Twin-Strep-tag are not accessible: Fuse tag with the other protein terminus. Use another linker.
- The ligand is blocked by biotinylated proteins from the extract: Add avidin if biotin-containing extracts are to be purified. The biotin content of the soluble part of the total *E. coli* cell lysate is about 1 nmol per liter culture (OD 550 = 1.0). Add 2 to 3 nmol of avidin monomer per nmol of biotin.

Contaminating proteins

- Contaminants are short forms of the tagged protein: Use protease deficient *E. coli* expression strains. Add protease inhibitors after cell lysis. Fuse the Strep-tag with the other protein terminus. Check for the presence of internal translation initiation starts (for C-terminal tag) or premature termination sites (for N-terminal *S*-tag). Use EDTA in the sample and buffers.
- Contaminants are non-covalently linked to the recombinant protein: Increase ionic strength in all buffers for cell lysis and purification or add mild detergents e.g., 0.1% Tween 20.

Unwanted air bubble formation

- Unclarified lysates may increase air bubble formation during purification. Attaching a flow restrictor in the chromatography system can prevent this. If possible, degas the sample using a vacuum degasser.
- Air bubble formation may occur due to decreased air solubility when columns stored at 4°C to 8°C are immediately used at room temperature. Let the columns adapt to room temperature before use.

8 Further information

Refer to IBA GmbH, Germany, www.iba-go.com, for expression, detection and/or assays for Strep-tag II and Twin-Strep-tagged recombinant proteins.

For further information, visit cytiva.com/hitrap or cytiva.com/protein-purification or contact your local Cytiva representative.

9 Ordering Information

Product	Pack size	Product code
StrepTrap XT	1 × 1 mL 5 × 1 mL 1 × 5 mL 5 × 5 mL	29401317 29401320 29401322 29401323

Related products	Pack size	Product code
Strep-Tactin XT Sepharose	10 mL	29401324
	50 mL	29401326
HiTrap Desalting	1 × 5 mL 5 × 5 mL	29048684 17140801
HiPrep 26/10 Desalting	1 × 53 mL 4 × 53 mL	17508701 17508702
PD-10 Desalting Columns	30	17085101

Accessories	Pack size	Product code
1/16" male/luer female 2 18111251 (For connection of syringe to top of HiTrap column)	2	18111251
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap* column)	2	18100368
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18101798

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