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Strep-Tactin XT **Sepharose**

Affinity media

Instructions for Use

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Abstract

Strep-Tactin™ XT Sepharose™ is a chromatography 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 ligand on the matrix is a specially engineered streptavidin. 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.

Strep-Tactin XT Sepharose is available in 10 and 50 mL lab packs and prepacked in 1 and 5 mL StrepTrap™ XT columns.

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1 Product description

This robust, high-resolution resin is based on the 34 µm Sepharose High Performance matrix. Due to the small size of the beads the bound recombinant protein is eluted in a narrow peak minimizing the need for further concentration steps.

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.

Table 1 summarizes the characteristics of Strep-Tactin XT Sepharose chromatography resin.

Table 1. Strep-Tactin XT Sepharose chromatography resin characteristics

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
Max. linear flow rate[2]	300 cm/h
Recommended linear flow rate[2]	≤ 150 cm/h
Maximum back pressure[2]	0.3 MPa (3 bar, 43.5 psi)
Chemical stability	Stable in all commonly used aqueous buffers (see Table 2).
pH, working range	pH 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 2)

Table 2. 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

The Strep-tag II is a small tag of only 8 amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) and it has 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 Column packing

Strep-Tactin XT Sepharose is supplied preswollen in 20% ethanol.

Prepare a slurry by decanting the 20% ethanol solution and replacing it with distilled water in a ratio of 75% settled resin to 25% distilled water. Water is used as packing solution.

Table 3. Recommended lab-scale columns for Strep-Tactin XT Sepharose

Empty Column[1]	**Packing flow rate (mL/min)**		**Recommended flow rate**[2] **for chromatography**
	First step	**Second step**	**(mL/min)**
Tricorn™ 5/20	0.5	1	0.5
Tricorn 5/50	0.5	1	0.5
Tricorn 10/20	2	4	2
Tricorn 10/50	2	4	2
Tricorn 10/100	2	4	2
XK 16/20	5	10	5
XK 26/20	13	27	13

[1] For inner diameter and maximum bed volumes and bed heights, see Section 9.
[2] The recommended flow rates equals a linear flow rate of approximately 150 cm/h.

Packing protocol

1. Assemble the column and packing reservoir if necessary.
2. Remove air from the end-piece by flushing with water. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.
3. Resuspend the resin and pour the slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.
4. Immediately fill the remainder of the column, or packing reservoir, with water. Mount the adapter or lid and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
5. Open the bottom outlet of the column and set the pump to run at the desired flow rate, see Table 3. It is recommended to pack Sepharose HP resin in XK or Tricorn columns in a two-step procedure.

   Note: *For subsequent chromatography procedures, do not exceed 75% of the packing flow rate. See Table 3 for flow rates for chromatography.*
6. Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.
7. Stop the pump and close the column outlet.
8. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.
9. With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.
10. Connect the column to the pump and start the flow. Re-adjust the adapter if necessary.

4 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. For example, 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: *Recommended flow velocity is 150 cm/h.*

1 Remove the stoppers and connect the column to the system.

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.*

2 Wash with 5 column volumes (CV) of distilled water at a flow velocity of 50-100 cm/h to remove ethanol.

3 Equilibrate with at least 5 CV binding buffer.

4 Load the sample.

5 Wash the column with 5 to 10 CV binding buffer or until no material appears in the effluent.

6 Elute with 6 CV elution buffer.

Regeneration

Note: *Recommended flow velocity is 150 cm/h.*

1. Regenerate the column with 3 CV distilled water followed by 50 mM NaOH during 3 min, and finally 3 CV distilled water.
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 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).

5 Scale-up

Scale-up is typically performed by keeping bed height and flow velocity (cm/h) constant while increasing bed diameter and flow rate (mL/min).

6 Storage

Store Strep-Tactin XT Sepharose 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 Strep-Tactin XT Sepharose, visit cytiva.com/hitrap or contact our technical support or your local Cytiva representative.

Fault	**Possible cause/*corrective action***
High back pressure	High viscosity of solutions or sample. • *Use lower flow rates.* • *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.* • *If the purification has been performed at 4°C, try repeating it at room temperature if possible (sample viscosity is reduced at room temperature).* Insufficient cell disruption. • *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).* Freezing/thawing of the lysate has increased precipitation and aggregation. • *Sonicating the thawed lysate can prevent increased backpressure problems when loading on the column.* • *Centrifuge and filter the sample through a 0.22 µm or a 0.45 µm filter.*
Column has clogged	Top filter is clogged. • *Change top filter.* Cell debris in the sample has clogged the column. • *Clean the column according to the section under Operation.* • *Centrifuge and filter the sample through a 0.22 µm or a 0.45 µm filter.*

Fault	Possible cause/*corrective action*
No or weak binding to the column	Protein found in the flow-through. • *Buffer/sample composition is not optimal; check the pH and composition of the sample and binding buffer. pH should be above 6.*
	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 Strep-tag II 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.*
	Protein has precipitated in the column due to high protein concentration. • *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.*
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 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 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 detergent, 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.*

Fault	Possible cause/*corrective action*
	Air bubbles may form due to decreased air solubility when columns stored at 4 to 8°C are used immediately at room temperature. • *Let the columns adapt to room temperature for some minutes before using them.*

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/protein-purification or our technical support portal *cytiva.com/support* or your local Cytiva representative.

9 Ordering Information

Product	Pack size	Product code
Strep-Tactin XT Sepharose	10 mL	29401324
Strep-Tactin XT Sepharose	50 mL	29401326

Related products	Pack size	Product code
StrepTrap XT	1 × 1 mL	29401317
	5 × 1 mL	29401320
	1 × 5 mL	29401322
	5 × 5 mL	29401323
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702
PD-10 Desalting Column	30	17085101
PD MiniTrap™ G-25	50	28918007
PD MidiTrap™ G-25	50	28918008

Empty lab-scale columns	Pack size	Product code
Tricorn 5/20 column, 5 mm i.d., max 0.55 mL bed volume or 2.8 cm bed height	1	28406408
Tricorn 5/50 column, 5 mm i.d., max 1.1 mL bed volume or 5.8 cm bed height	1	28406409
Tricorn 10/20 column, 10 mm i.d., max 2.2 mL bed volume or 2.8 cm bed height	1	28406413
Tricorn 10/50 column, 10 mm i.d., max 4.5 mL bed volume or 5.8 cm bed height	1	28406414
Tricorn 10/100 column, 10 mm i.d., max 8.5 mL bed volume or 10.8 cm bed height	1	28406415
XK 16/20 column, 16 mm i.d., max 30 mL bed volume or 15 cm bed height	1	28988937
XK 26/20 column, 26 mm i.d., max 65 mL bed volume or 12.5 cm bed height	1	28988948

Related literature	Product code
Affinity Chromatography Handbook, Principles and Methods	18102229
Affinity Chromatography Column and Media, Selection Guide	18112186
Recombinant Protein Purification Handbook, Principles and Methods	18114275

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