Strep-Tactin XT **Sepharose** affinity chromatography resin **StrepTrap** XT prepacked columns

AFFINITY CHROMATOGRAPHY

Strep-Tactin® XT Sepharose™ is a chromatography resin for purification of recombinant proteins tagged with Strep-tag® II and Twin-Strep-tag®. These proteins bind very specifically to the immobilized Strep-Tactin XT ligand giving highly pure target protein. Strep-Tactin XT Sepharose contains the latest Strep-Tactin ligand, XT, which provides higher affinity, resulting in higher yields and lower loss of valuable target protein than with the predecessor product, StrepTactin Sepharose High Performance (HP). Strep-Tactin XT Sepharose also enables purification under denaturing conditions.

Strep-Tactin XT Sepharose benefits:

- Highly pure Strep-tag II and Twin-Strep-tag recombinant proteins
- Fast purifications with simple elution
- Physiological conditions and mild elution preserve target protein activity
- Fast and easy regeneration
- Compatible with a wide range of detergents, denaturants, and other additives
- Available in 10 and 50 mL lab packs of bulk resin and prepacked in 1 and 5 mL StrepTrap™ XT chromatography columns for convenience

Fig 1. Strep-Tactin XT Sepharose resin and StrepTrap XT prepacked columns give fast and convenient affinity purification of recombinant proteins tagged with Strep-tag II and Twin-Strep-tag.

Strep-tag II and Twin-Strep-tag recombinant proteins

Strep-tag II is a small tag consisting of only eight amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) and a molecular weight (M_r) of 1000. The small tag will generally not interfere with structural and functional studies, which minimizes the need for tag removal after purification. Twin-Strep-tag is a sequential arrangement of two Strep-tag II sequences with increased affinity for Strep-Tactin XT Sepharose.

Chromatography resin characteristics

The ligand on Strep-Tactin XT Sepharose is engineered from streptavidin with high binding affinity in the low picomolar (pM) range for Twin-Strep-tag.

The ligand is immobilized on Sepharose HP base matrix. Due to the small size of the beads (34 µm), the bound recombinant protein is eluted in a narrow peak, minimizing the need for further concentration steps.

Strep-Tactin XT Sepharose is compatible with a wide range of additives and is easily regenerated using 0.05 M NaOH. Up to 0.5 M NaOH can be used for cleaning the column. Note that incubation with NaOH for long periods of time can permanently reduce binding capacity of the resin. See product instructions 29401327 for guidelines.

StrepTrap XT prepacked column characteristics

StrepTrap XT columns are prepacked with Strep-Tactin XT Sepharose. The prepacked columns are 1 mL and 5 mL HiTrap™ columns made of biocompatible polypropylene that does not interact with biomolecules. Prepacked StrepTrap XT columns provide fast and simple purifications in a convenient format.

The columns are delivered with a stopper on the inlet, a snap-off end on the outlet, and porous top and bottom frits allow high flow rates.

Note that HiTrap columns cannot be opened or refilled.

Tables 1 and 2 summarize the characteristics of Strep-Tactin XT Sepharose and StrepTrap XT columns, respectively. Table 3 lists the compatibility of the Strep-Tactin XT Sepharose resin with different additives.

Table 2. Characteristics of StrepTrap XT columns

Column volumes	1 mL or 5 mL
Column dimensions	0.7 × 2.5 cm (1 mL) 1.6 × 2.5 cm (5 mL)
Recommended operating flow rates[1]	1 and 5 mL/min for 1 and 5 mL columns, respectively
Maximum operating flow rates[1]	4 and 20 mL/min for 1 and 5 mL columns, respectively
Column hardware pressure limits	0.5 MPa (5 bar, 70 psi)

[1] At room temperature using a buffer with the same viscosity as water.

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.

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%
Sodium-N-dodecyl sulfate (SDS)[2]	0.09%
Glycerol	25%
Ethanol	10%
Imidazole	250 mM

[1] Data kindly provided by IBA GmbH, 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%.

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 of the protein, the possible concentration may deviate from the given value between proteins.

Note: Do not include dithiothreitol (DTT) in buffers.

Table 1. Characteristics of Strep-Tactin XT Sepharose

Matrix	Rigid, cross-linked agarose, spherical
Particle size, d_{50v}[1]	34 µm
Ligand	Strep-Tactin XT
Ligand concentration	~ 5 mg/mL resin
Dynamic binding capacity, Q_{b10}[2]	~ 10 mg protein with Strep-tag II or Twin-Strep-tag/mL of resin
Recommended maximum operating flow velocity[3]	300 cm/h
Recommended operating flow velocity[3]	≤ 150 cm/h
Maximum back pressure	0.3 MPa (3 bar, 43.5 psi)
Chemical stability	Stable in all commonly used aqueous buffers (see Table 3)
pH stability, operational[4]	pH 6 to 10
Storage	2°C to 8°C in 20% ethanol

[1] Median particle size of the cumulative volume distribution.
[2] Binding capacity is protein dependent. Dynamic binding capacity (DBC) is defined as milligram of protein applied per milliliter of resin at the point where the concentration of protein in the column eluent 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 StrepTrap XT column (1 min residence time) for GAPDH Twin-Strep-tag (M_r 39 400/subunit) and GAPDH Strep-tag II (M_r 37 400/subunit) in 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.
[3] At room temperature using a buffer with the same viscosity as water.
[4] pH range where resin can be operated without significant change in function.

Use and application

Packing in laboratory columns

Strep-Tactin XT Sepharose is supplied preswollen in 10 mL and 50 mL packs. The resin is easy to pack and use in, for example, laboratory columns from the Tricorn™ and XK series (*see* Ordering information). Full user instructions are supplied with each pack.

StrepTrap XT prepacked columns

Purifications on StrepTrap XT 1 mL and 5 mL are easily performed using a syringe and the provided Luer adapter, a laboratory pump, or an ÄKTA™ liquid chromatography system. Full user instructions are supplied with each pack.

Comparison of Strep-Tactin XT Sepharose and StrepTactin Sepharose HP

Strep-Tactin XT Sepharose is the successor of StrepTactin Sepharose HP, offering higher affinity and enabling purification under denaturing conditions. The higher affinity gives higher protein yields and minimizes loss of valuable target protein.

To demonstrate this, purification of 3 mg GAPDH Strep-tag II spiked in *E. coli* lysate was performed on both StrepTrap XT and StrepTrap HP 1 mL columns. Figure 2 shows the chromatograms and running conditions. Absorbance measurements of the eluted pools revealed that 98% of the target protein was recovered using StrepTrap XT whereas only 84% was recovered using StrepTrap HP. SDS-PAGE (Fig 2C) showed that the purity of the eluted pools was high using either column.

Columns:	StrepTrap XT 1 mL StrepTrap HP 1 mL
Sample:	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH Strep-tag II, tetramer, M_r 37 400/subunit), 1 mg/mL in *E. coli* lysate
Sample volume:	3 mL
Binding buffer:	100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0
Elution buffer:	StrepTrap XT column: 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 50 mM biotin, pH 8.0 StrepTrap HP column: 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, pH 8.0
Regeneration:	StrepTrap XT column: 3 column volumes (CV) distilled water, 3 CV 50 mM NaOH, 3 CV distilled water StrepTrap HP column: 3 CV distilled water, 3 CV 500 mM NaOH, 3 CV distilled water
Flow rate:	1 mL/min
System:	ÄKTA pure 25

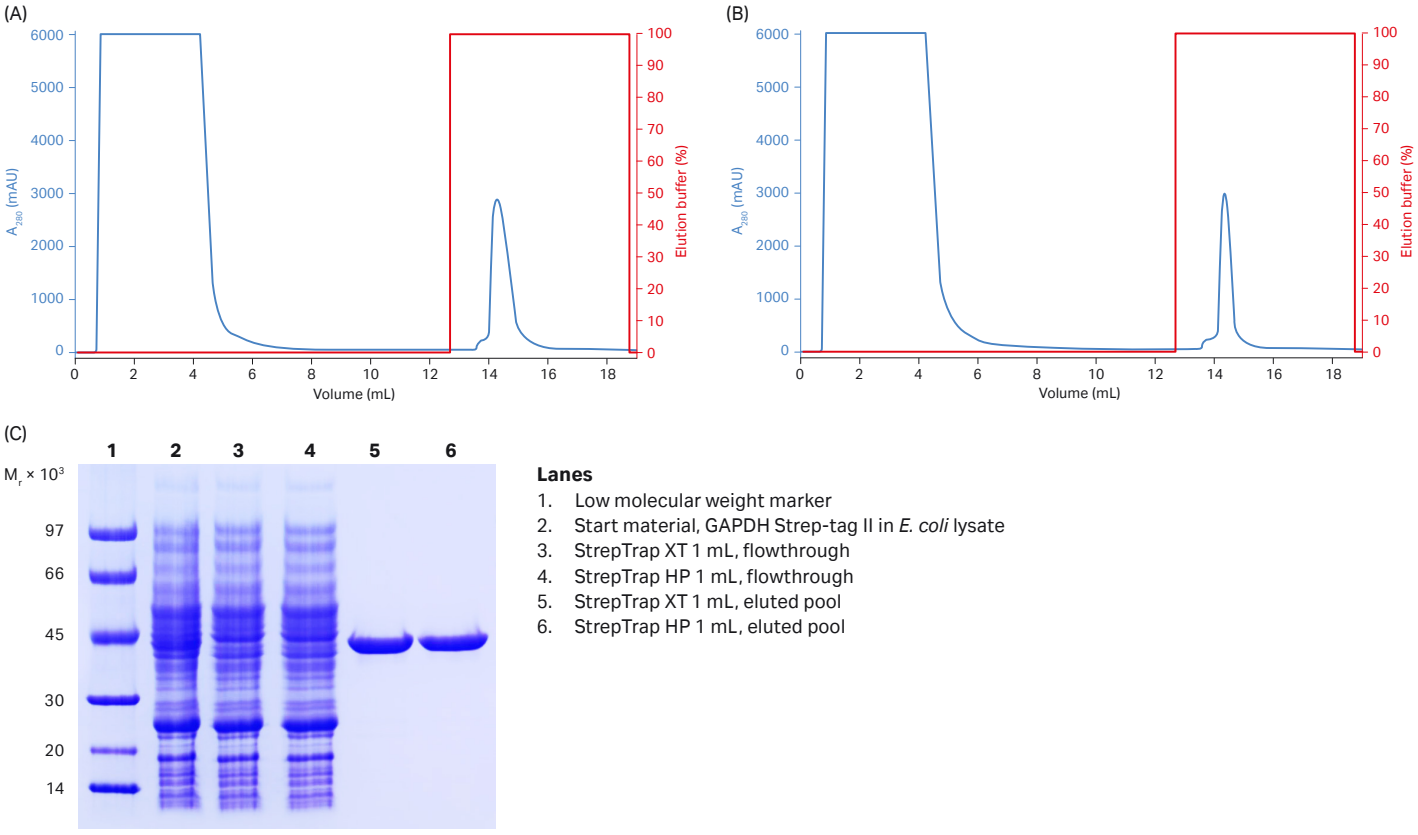
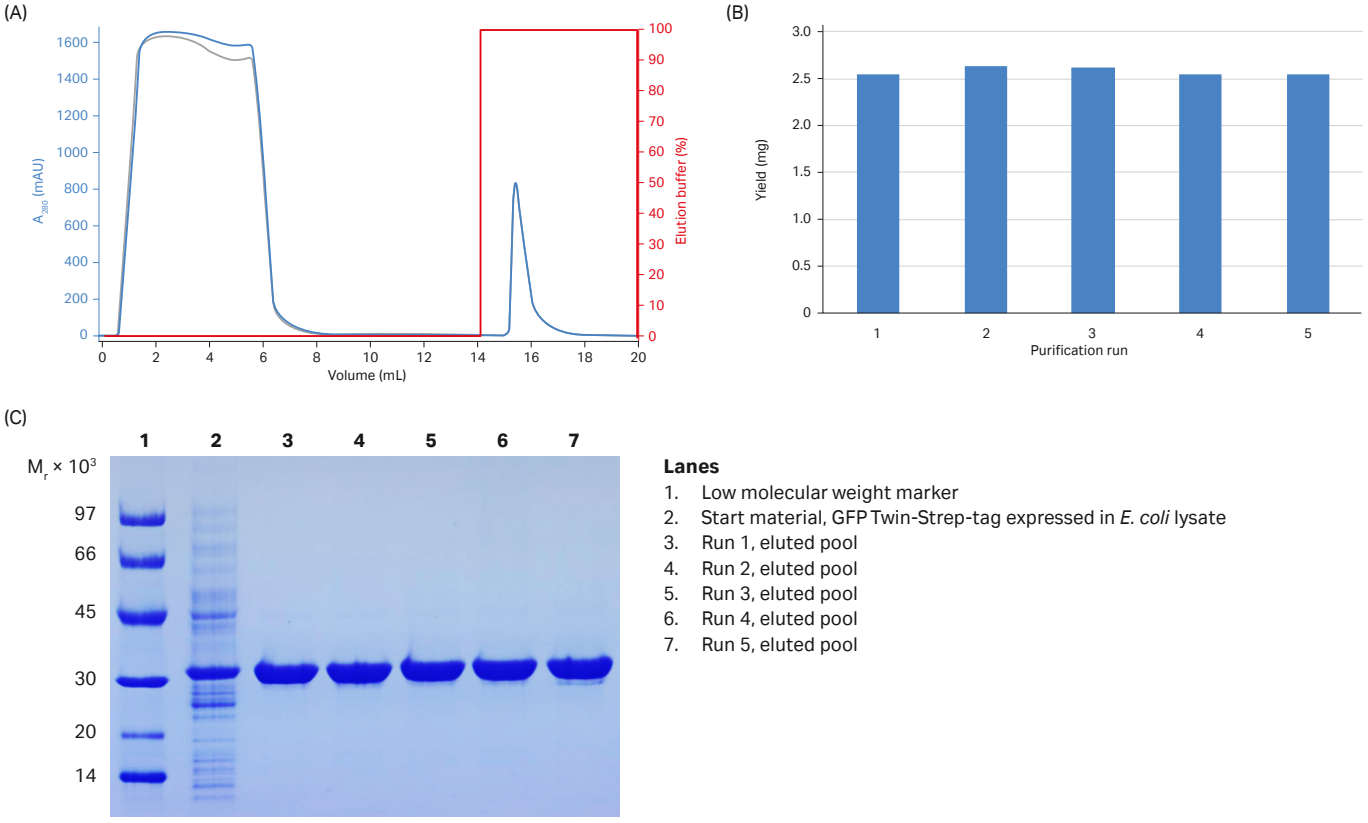


Fig 2. Purification of 3 mg of GAPDH Strep-tag II on (A) StrepTrap XT 1 mL and (B) StrepTrap HP 1 mL. (C) SDS-PAGE analysis (reduced conditions, Coomassie™ blue stained) comparing purification on the two columns.

Repeatable purifications using StrepTrap XT column

Purifications using StrepTrap XT may be performed with high repeatability. To demonstrate this, GFP Twin-Strep-tag (M_r 30 200) was purified on StrepTrap XT 1 mL. Five repetitive runs were made with regeneration using 50 mM NaOH between each run. Figure 3A shows an overlay of the chromatograms from run 1 and 5, illustrating the very high reproducibility of StrepTrap XT columns. Comparable yield was obtained in all runs (Fig 3B) and the purity was high and very consistent throughout the entire study (Fig 3C).

Column:	StrepTrap XT 1 mL
Sample:	Twin-Strep-tagged green fluorescent protein (GFP Twin-Strep-tag, M_r 30 200) expressed in *E. coli* lysate
Sample volume:	5 mL
Binding buffer:	100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0
Elution buffer:	100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 50 mM biotin, pH 8.0
Regeneration:	3 CV of distilled water, 3 CV of 50 mM NaOH, 3 CV of distilled water
Flow rate:	1 mL/min
System:	ÄKTA go



Lanes

1. Low molecular weight marker
2. Start material, GFP Twin-Strep-tag expressed in *E. coli* lysate
3. Run 1, eluted pool
4. Run 2, eluted pool
5. Run 3, eluted pool
6. Run 4, eluted pool
7. Run 5, eluted pool

Fig 3. Five GFP Twin-Strep-tag purification runs on the same StrepTrap XT 1 mL column. (A) Overlay of chromatograms from run 1 (blue) and run 5 (gray). (B) Graph showing eluted yield. (C) SDS-PAGE analysis of eluted pools (reduced conditions, Coomassie blue stained).

Scaling up

Scale-up can be achieved by increasing the bed volume while keeping the residence time constant. This approach maintains chromatographic performance during scale-up.

For quick scale-up, two or more StrepTrap XT columns can be connected in series. Note, however, that this may increase back pressure on the column.

The scale-up study below describes the approch of increasing the bed volume. The protein used was a recombinant protein with Strep-tag II, GAPDH Strep-tag II, in *E. coli* lysate. Purification on a StrepTrap XT 1 mL column was first performed and then scaled up to the 5 mL column. The protein load was increased five-fold and the residence time was 1 min for both columns.

Figure 4 A and B show the chromatograms and running conditions. Yield, calculated from absorbance measurements, was 5.5 and 29.2 mg, respectively (Table 4). SDS-PAGE (Fig 4C) showed that the purity of the fractions eluted from the columns was high in both runs.

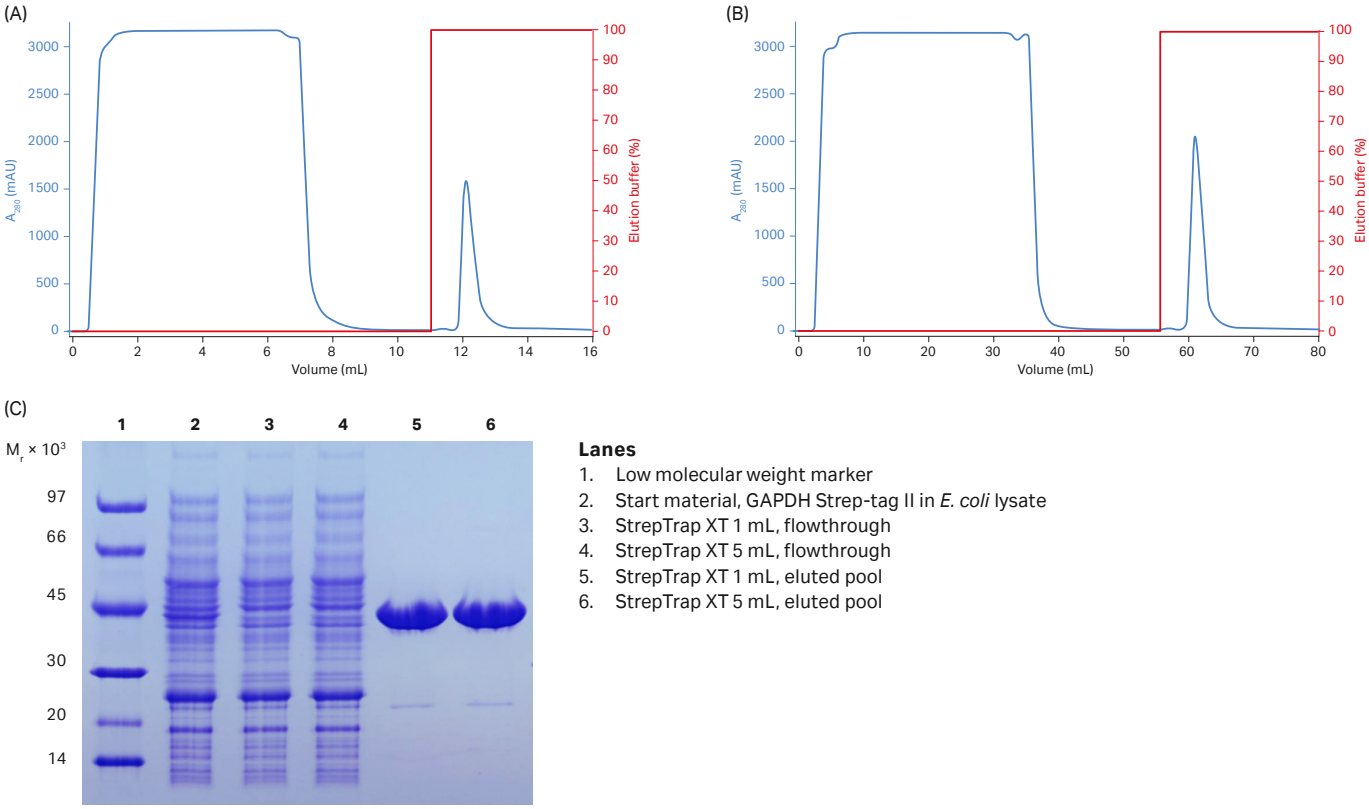
The columns gave comparable results, confirming the ease and reproducibility of scaling up purifications from StrepTrap XT 1 mL to StrepTrap XT 5 mL.

If a larger column is needed, Strep-Tactin XT Sepharose can easily be packed in, for example, a column from the Tricorn or XK series.

Table 4. Overview of the yield for StrepTrap XT 1 and 5 mL

Column	Yield (mg)
StrepTrap XT, 1 mL	5.5
StrepTrap XT, 5 mL	29.2

Columns:	StrepTrap XT 1 mL StrepTrap XT 5 mL
Sample:	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH Strep-tag II, tetramer, M_r 37 400/subunit), 1 mg/mL in *E. coli* lysate
Sample volumes:	6 mL (StrepTrap XT 1 mL) 30 mL (StrepTrap XT 5 mL)
Binding buffer:	100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0
Elution buffer:	100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 50 mM biotin, pH 8.0
Regeneration:	3 CV distilled water, 3 CV 50 mM NaOH, 3 CV distilled water
Flow rates:	1 mL/min (StrepTrap XT 1 mL) 5 mL/min (StrepTrap XT 5 mL)
System:	ÄKTA go



Lanes

1. Low molecular weight marker
2. Start material, GAPDH Strep-tag II in *E. coli* lysate
3. StrepTrap XT 1 mL, flowthrough
4. StrepTrap XT 5 mL, flowthrough
5. StrepTrap XT 1 mL, eluted pool
6. StrepTrap XT 5 mL, eluted pool

Fig 4. Scaling up the purification of GAPDH Strep-tag II from (A) StrepTrap XT 1 mL to (B) StrepTrap XT 5 mL. (C) SDS-PAGE analysis (reduced conditions, Coomassie blue stained).

Further information

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

Ordering information

Product	Pack size	Product code
Strep-Tactin XT Sepharose	10 mL	29401324
	50 mL	29401326
StrepTrap XT	1 × 1 mL	29401317
	5 × 1 mL	29401320
	1 × 5 mL	29401322
	5 × 5 mL	29401323
Empty columns		
Tricorn 5/20 column (max. 0.55 mL or 2.8 cm)	1	28406408
Tricorn 5/50 column (max. 1.1 mL or 5.8 cm)	1	28406409
Tricorn 10/20 (max. 2.2 mL or 2.8 cm)	1	28406413
Tricorn 10/50 (max. 4.5 mL or 5.8 cm)	1	28406414
Tricorn 10/100 (max. 8.5 mL or 10.8 cm)	1	28406415
XK 16/20 (max. 30 mL or 15 cm)	1	28988937
XK 26/20 (max. 65 mL or 12.5 cm)	1	28988948
Accessories		
1/16" male/Luer female (or 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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