Strep•Tactin® Purification Kits

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About the Kits

Product	Size	Cat. No.
Strep•Tactin® Superflow™ Agarose	2 ml	71592-3
	10 ml	71592-4
Strep•Tactin Superflow Column, 0.2 ml	5 columns	71594-3
Strep•Tactin Superflow Column, 1 ml	5 columns	71593-3
Strep•Tactin Superflow Cartridge, 1 ml	5 cartridges	71595-3
Strep•Tactin Superflow Cartridge, 5 ml	1 cartridge	71596-3
Strep•Tactin Superflow Cartridge, 5 ml	5 cartridges	71596-4
Strep•Tactin MacroPrep® Agarose	2 ml	71597-3
	10 ml	71597-4
Strep•Tactin MacroPrep® Cartridge, 1 ml	5 cartridges	71598-3
Introductory Strep•Tag® II System, pET-51	1 kit	71615-3
Introductory Strep•Tag® II System, pET-52	1 kit	71616-3
Strep•Tactin Buffer Kit	1 kit	71613-3

Description

Strep•Tag® technology is based on the strong and specific interaction between biotin and streptavidin. To take advantage of this interaction for recombinant protein purification, the 8-amino acid Strep•Tag II fusion tag was developed to bind to the biotin binding pocket of streptavidin. Likewise, the Strep•Tactin protein is a streptavidin derivative developed for optimal Strep•Tag II binding. The binding affinity of Strep•Tag II for Strep•Tactin is approximately 100 times higher than for streptavidin.

The Strep•Tactin family of products offers a variety of resins for rapid one-step affinity purification of proteins containing the Strep•Tag II fusion tag. The Strep•Tag II sequence binds to the Strep•Tactin, and after unbound proteins are washed away, the purified target protein is competitively eluted with 2.5 mM desthiobiotin, an analog of biotin that reversibly binds Strep•Tactin. The purification steps may be performed in PBS or other physiological buffers.

Strep•Tactin Superflow Agarose

Strep•Tactin Superflow Agarose is a cross-linked agarose derivatized with Strep•Tactin protein. The binding capacity is 50-100 nmol/ml settled resin, or up to 3 mg of 30 kDa protein per ml settled resin. It can be used for gravity flow as well as for low pressure and FPLC chromatography. Strep•Tactin Superflow Agarose is optimized for column affinity chromatography and is not recommended for batch purification. It is supplied as a 50% slurry.

Strep•Tactin Superflow Column

Strep•Tactin Superflow Columns are ready-to-use prepacked chromatography columns designed for gravity flow. Columns are available in two sizes, 0.2 ml and 1 ml, with binding capacities of 10-20 nmol/column and 50-100 nmol/column, respectively.

Strep•Tactin Superflow Cartridge

Strep•Tactin Superflow Cartridges provide the ultimate convenience for affinity purification by low pressure chromatography. The luer lock fittings fasten quickly to a syringe or connect several cartridges in a series to allow for higher purification capacities. Adaptors are available separately for use with liquid chromatography systems or FPLC workstations. The cartridges are available in two sizes, 1 ml and 5 ml, with binding capacities of 50-100 nmol/cartridge and 250-500 nmol/cartridge, respectively.

Strep•Tactin® MacroPrep® Resin

Strep•Tactin MacroPrep Resin is a polymethacrylate resin suitable for gravity flow and all low pressure chromatography applications. This resin exhibits non-specific binding properties that differ from the Strep•Tactin Superflow™ Agarose and therefore is recommended when suboptimal results are obtained with Superflow. The binding capacity is 50–100 nmol/ml settled resin or up to 3 mg of 30 kDa protein per ml settled resin. Strep•Tactin MacroPrep Resin is optimized for column affinity chromatography and is not recommended for batch purification. It is supplied as 50% slurry.

Strep•Tactin MacroPrep Cartridge

Strep•Tactin MacroPrep Cartridges provide the ultimate convenience for affinity purification by low pressure chromatography. The luer lock fittings fasten quickly to a syringe or connect several cartridges in a series to allow for higher purification capacities. Adaptors are available separately for use with liquid chromatography systems. Each 1ml cartridge has a binding capacity of 50-100 nmol.

Strep•Tactin Buffer Kit

The Strep•Tactin Buffer Kit is a set of pre-tested buffers designed for use with Strep•Tactin resins for convenient, rapid one-step purification of the Strep•Tag® fusion proteins. The kit includes Strep•Tactin Wash, Elution, and Regeneration buffers.

Components

Strep•Tactin Buffer Kit

- 100 ml 10X Strep•Tactin Wash Buffer (1.5 M NaCl, 1 M Tris-HCl, 10 mM EDTA, pH 8.0)
- 25 ml 10X Strep•Tactin Elution Buffer (1.5 M NaCl, 1 M Tris-HCl, 10 mM EDTA, 25 mM desthiobiotin, pH 8.0)
- 100 ml 10X Strep•Tactin Regeneration Buffer (1.5 M NaCl, 1 M Tris-HCl, 10 mM EDTA, 10 mM HABA (hydroxy-azophenyl-benzoic acid, pH 8.0)

Introductory Strep•Tag II System, pET-51

- 10 µg pET-51b(+) DNA
- 1 × 1 ml Strep•Tactin Superflow Column
- 120 ml 1X Strep•Tactin Wash Buffer (150 mM NaCl, 100 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 25 ml 1X Strep•Tactin Elution Buffer (150 mM NaCl, 100 mM Tris-HCl, 1 mM EDTA, 2.5 mM desthiobiotin, pH 8.0)
- 120 ml 1X Strep•Tactin Regeneration Buffer (150 mM NaCl, 100 mM Tris-HCl, 1 mM EDTA, 1 mM HABA (hydroxy-azophenyl-benzoic acid, pH 8.0)
- 20 µ1 Strep•Tag II Antibody, HRP Conjugate
- 0.2 ml Induction Control D Glycerol Stock

Introductory Strep•Tag II System, pET-52

- 10 µg pET-52b(+) DNA
- 1 × 1 ml Strep•Tactin Superflow Column
- 120 ml 1X Strep•Tactin Wash Buffer (150 mM NaCl, 100 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 25 ml 1X Strep•Tactin Elution Buffer Buffer (150 mM NaCl, 100 mM Tris-HCl, 1 mM EDTA, 2.5 mM desthiobiotin, pH 8.0)
- 120 ml 1X Strep•Tactin Regeneration Buffer (150 mM NaCl, 100 mM Tris-HCl, 1 mM EDTA, 1 mM HABA (hydroxy-azophenyl-benzoic acid, pH 8.0)
- 20 µl Strep•Tag II Antibody, HRP Conjugate
- 0.2 ml Induction Control D Glycerol Stock

Available separately

FPLC adaptor sets (M6, ¼-28, 10-32, 1/16) are available separately. Strep•Tactin Elution, Wash, Regeneration, and Biotin Blocking Buffers are also available separately.

Storage

Store Strep•Tactin Superflow Agarose, Strep•Tactin Superflow Columns, Strep•Tactin Superflow Cartridges, Strep•Tactin MacroPrep Agarose, Strep•Tactin MacroPrep Cartridges, 10X Strep•Tactin Wash Buffer, 10X Strep•Tactin Regeneration Buffer, 10X Strep•Tactin Elution Buffer, Strep•Tag II Monoclonal Antibody at 4°C. Store pET-51b(+) and pET-52 b(+) DNA at –70°C.

Overview

This technical bulletin describes methods for *E. coli* cell extract preparation, and procedures for purification of protein with a Strep•Tag II sequence using the Strep•Tactin® supports. These procedures begin with cell culture that has been induced for target protein production. The fusion protein should contain an 8-amino acid Strep•Tag II fusion tag at the N-terminus or C-terminus of the polypeptide. A detailed discussion of target protein induction using the Novagen pET System can be found in the pET System Manual User Protocol TB055, which accompanies pET vectors and systems, and is also available at www.novagen.com. Information is also available for the Novagen pIEx™ and pTriEx™ systems (see User Protocols TB357 and TB250, respectively).

For proteins expressed using the pET System, a yield of 20 mg target protein per 100 ml culture is not unusual. The binding capacity of the matrix is 1–2 mg recombinant protein (50–100 nmol/ml of a 20 kDa protein) per ml bed volume. Like other affinity chromatography methods, the highest purity of target proteins is achieved when using amounts of extract and resin such that the resin will be near its binding capacity. Thus, we recommend obtaining an estimate of the quantity of target protein present in the extract. SDS-PAGE, or other protein-specific assays can estimate the mass of target protein in crude extracts. Once an estimate of the quantity of target protein has been determined, prepare a corresponding amount of resin, or choose the appropriately sized pre-packed column or cartridge.

Considerations before you begin

- In the following protocol, one volume is equivalent to the settled bed volume (e.g. 100 µl slurry yields 50 µl resin for a settled bed volume of 50 µl).
- If working with metalloproteins, use buffers without EDTA.
- Purification is performed at 4–30°C, according to the temperature stability of the target protein.
- Modification of the Strep•Tactin Wash, and Elution Buffers is possible. See Table 1 for a list of components that have been evaluated successfully with the Strep•Tactin system.
- Add avidin (Biotin Blocking Buffer, Cat. No. 71609), if biotin containing extracts are to be purified. The soluble fraction of E. coli lysate contains approximately 1 nmol biotin/ L culture. Add 2–3 nmol avidin/ nmol biotin.

Table 1

Condition or Component	Upper limit evaluated
Ammonium Sulfate	2 M
$CaCl_2$	1 M
EDTA*	50 mM
Ethanol	10%
Guanidine	1 M
Glycerol	25%
Imidazole	250 mM
$MgCl_2$	1 M
NaCl	5 M
Urea	1 M
CHAPS	0.1%
N-lauryl-sarcosine	2%
SDS*	0.1%
DTT*	50 mM
β–mercaptoethanol*	50 mM
Triton®X-100	2%
TWEEN® 20	2%

*While the concentration of these reagents is compatible with the Strep•Tactin system, compatibility with Ni-NTA His•Bind resin may be affected.

Extract preparation

The volume of the cell extract should be between 0.5 and 10 vol relative to the bed volume of the column. Extracts with low target protein concentration may lead to reduced recovery yield. We recommend protein concentrations of between 50 and 100 nmol recombinant Strep•Tag II fusion protein per 1 ml column.

Bacterial

Extracts can be prepared using BugBuster™ Protein Extraction Reagent (see User Protocol TB245), standard mechanical lysis protocols, or enzymatic methods, including rLysozyme™ (see User Protocol TB334) and Benzonase Nuclease (see User Protocol TB261), or Lysonase™ Bioprocessing Agent (see User Protocol TB361).

Insect

Extracts can be prepared using CytoBuster™ Protein Extraction Reagent (see User Protocol TB306), or standard mechanical lysis protocols.

Mammalian

Extracts can be prepared using CytoBuster™ Protein Extraction Reagent, or standard mechanical lysis protocols.

Note: If sample is stored between extract preparation and purification, centrifuge extract at 14,000 × g for 5 min at 4°C, to remove aggregates that may have formed during storage.

Buffer preparation

1. Dilute an appropriate amount supplied stock 10X Strep•Tactin® Wash Buffer to 1X with distilled water.
2. Dilute an appropriate amount supplied stock 10X Strep•Tactin Elution Buffer to 1X with distilled water.

Note: Modification of the Strep•Tactin Wash, and Elution Buffers is possible. pH should not be lower than 7. See Table 1 for a list of components that have been evaluated successfully with the Strep•Tactin system.

Strep•Tactin Column Chromatography

Column preparation

Strep•Tactin Superflow Agarose and Strep•Tactin MacroPrep Resin

Small polypropylene columns, such as the Novagen Chromatography Columns (Cat. No. 69673), that hold 2.5 ml settled resin, can be used.

Note: Adding a few ml sterile, deionized water to the dry column, and gently pushing on the column top with a gloved finger, will wet the frit and start column flow.

1. Gently mix bottle of Strep•Tactin Superflow Agarose by inversion until completely suspended. Using a wide-mouth pipet, transfer desired amount of slurry to column (e.g. 100 µl slurry yields 50 µl resin for settled bed volume of 50 µl). Allow resin to pack under gravity flow.
2. When level of storage buffer drops to top of column bed, equilibrate column with 2 vol 1X wash buffer. Allow entire buffer volume to flow through column.

Column preparation

Strep•Tactin Superflow Column

1. Remove top column cap.
2. Remove lower luer plug.
3. Allow excess storage buffer to drain.

Note: The column will not run dry under gravity flow.

4. Equilibrate column with 2 vol 1X wash buffer. Allow entire buffer volume to flow through column.

Column chromatography

1. After wash buffer has drained, load column with prepared cell extract.

Note: Apply extract to column as concentrated as possible in recommended volume range.

2. Wash column 5 times, each with 1 vol 1X wash buffer.
3. Elute recombinant protein from resin 6 times, each with 0.5 vol 1X elution buffer. The target protein generally elutes off column between the third and fifth fractions.
4. Collect eluate in fractions for SDS-PAGE analysis.

Strep•Tactin® Cartridges Protocol

For using a 1-ml or 5-ml cartridge with a syringe.

Cartridge preparation

Strep•Tactin Superflow® or MacroPrep® Cartridges

1. Fill cartridge inlet with 1X wash buffer.
2. Fill 10-ml or 30-ml syringe with 1X wash buffer.
3. Connect cartridge to syringe, avoiding air bubbles.
4. Equilibrate column with 5 vol 1X wash buffer at a rate of 1 drop/s.

Cartridge chromatography

1. Remove syringe used for equilibration from cartridge.
2. Fill cartridge inlet with 1X wash buffer.
3. Connect syringe containing cell extract to cartridge. Load cell extract onto column at a rate of 1 drop/2 s.
4. Remove syringe from cartridge.
5. Fill cartridge inlet with 1X wash buffer.
6. Fill 10-ml syringe (for 1-ml cartridges) or 30-ml syringe (for 5-ml cartridges) with 1X wash buffer.
7. Connect syringe with 1X wash buffer to cartridge. Load 5 vol 1X wash buffer onto column at a rate of 1 drop/2 s.
8. Remove syringe from cartridge.
9. Fill cartridge inlet with 1X elution buffer.
10. Fill 5-ml or 30-ml syringe with 4 vol 1X elution buffer and connect to cartridge.
11. Load 4 ml elution buffer onto column at a rate of 1 drop/2 s.
12. Collect eluate in fractions for SDS-PAGE analysis.

Strep•Tactin Cartridges FPLC Protocol

Strep•Tactin Superflow or Strep•Tactin MacroPrep Cartridges

Cartridge chromatography

1. Connect appropriate adaptor to top of cartridge.
2. Equilibrate cartridge with 5 vol 1X wash buffer at a rate no greater than 1 ml/min for 1-ml cartridges and 3 ml/min for 5-ml cartridges.

Note: Monitor elution at 280 nm. The baseline should be stable after equilibration.

3. Load cell extract onto cartridge. Use an initial flow rate of 1ml/min.

Note: Lysate viscosity may cause the pressure to exceed 25 psi. If required, either reduce flow rate or reduce viscosity of cell extract using Benzonase Nucease (see User Protocol TB261).

4. Wash with 5–10 vol 1X wash buffer until A_{280} is stable. Proceed with Step 5 as soon as baseline is reached to achieve maximum protein yields.
5. Elute target from column with 1X elution buffer.
6. Collect eluate fractions for SDS-PAGE analysis.

Processing Sample after Elution

After eluting purified protein from a Strep•Tactin® support, the sample can be concentrated, or the buffer changed by one of several methods. The storage buffer for your purified protein is often determined through an empirical process. Inappropriate storage buffer may lead to precipitation of the protein. If necessary, desthiobiotin and EDTA can be removed by dialyis, filtration, or size exclusion chromatography.

1. Dialyze into buffer of choice.
2. Use the Novagen D-Tube™ Dialyzers (see User Protocol TB422) for dialysis and sample concentration.
3. Use plastic disposable microconcentrator units (e.g., Millipore; Pall), as directed by manufacturer, to both desalt and concentrate the sample by ultrafiltration.
4. Desalt sample by gel filtration on Sephadex (G-10, G-25, G-50; Pharmacia) or Bio-Gel (P6DGm P-10, P-30; Bio-Rad).

Resin regeneration

When elution is complete, Strep•Tactin Resin can be regenerated for reuse up to five times. However, because some small amounts of protein may not be released with treatment, we recommend a different sample of resin for each different protein studied.

1. Dilute an appropriate amount supplied stock 10X Strep•Tactin Regeneration Buffer to 1X with distilled water.
2. Dilute an appropriate amount supplied stock 10X Strep•Tactin Wash Buffer to 1X with distilled water.

Resin regeneration- agarose/resin/columns

1. Wash column 3 times, each with 5 vol 1X regeneration buffer.

Note: Regeneration is signaled by a color change from yellow to red. The intensity of the red color is an indication of the activity status of the resin.

2. Regeneration is complete when color intensity is uniform throughout the column. If regeneration is not complete, continue to add regeneration buffer until the uniformity of the red color is the same at the top of column as it is on the bottom of column.
3. Add 2 vol 1X wash buffer or 1X regeneration buffer for storage.
4. Store column at of 4–8°C.
5. Wash 2 times, each with 4 vol 1X wash buffer prior to use.

Resin regeneration- cartridges

1. Fill cartridge inlet with 1X regeneration buffer.
2. Fill 20-ml syringe with 1X regeneration buffer.
3. Load 15 vol regeneration buffer onto cartridge at a flow rate of 1 drop/s.
4. Regeneration is complete when color intensity is uniform throughout the column. If regeneration is not complete, continue to add regeneration buffer until the intensity of the red color is the same at the top of column as it is on the bottom of column.
5. Add 2 vol 1X wash buffer or 1X regeneration buffer for storage.
6. Store column at of 4–8°C.
7. Wash 2 times, each with 4 vol 1X wash buffer prior to use.

Troubleshooting guide

Problem	Probable Cause	Solution
No binding or inefficient binding to Strep•Tactin®Column	Strep•Tag II is not present.	Sequence ligation junctions to verify correct reading frame. Use protease deficient *E. coli* strains. Use protease inhibitors.
	Strep•Tag II is not accessible.	Incorporate a linker between target and tag. Engineer tag into other protein terminus.
	Strep•Tag II is partially accessible	Reduce washing to 3 vol wash buffer
	Strep•Tactin Column is inactive.	Evaluate with HABA. If biotin-containing extracts are to be used, add avidin* (Biotin Blocking Buffer).
	Incorrect pH	pH should be > 7.0
	Protein concentration too low	Concentrate protein prior to column chromatography.
Protein elutes with contaminants**	Contaminants are truncated forms of the target protein.	Use protease deficient *E. coli* strains. Use protease inhibitors. Engineer tag into C-terminus of protein. Use Rosetta™ strain if protein contains rare codons.
	Contaminants are co-purifying with target protein via disulfide bonds.	Add reducing agents to all buffers for cell lysis and chromatography to break disulfide bonds.
	Contaminants are co-purifying with target protein via non-covalent linkage.	Increase ionic strength (up to 5 M NaCl) of all buffers for cell lysis and chromatography; Add non-ionic detergents (up to 2% Triton®X-100, 2% TWEEN®, 0.1% CHAPS, etc.), to all buffers for cell lysis and chromatography.
Bubbles in column	Bubbles form in column when taken from cold storage.	Use degassed buffers; Continue work in cold room; Immediately wash column with buffers at ambient temperature once column is removed from the cold.

*The soluble fraction of the *E. coli* total cell lysate contains approximately 1 nmol biotin/L culture (OD_{550}= 1.0). Biotin concentrations may be much higher in cell culture supernatants. Add 2–3 nmol avidin monomer per nmol biotin.

**Except for BCCP (biotin carboxyl carrier protein), the soluble fraction of *E. coli* total cell extract contains no proteins that bind the Strep•Tactin resin. BCCP binds irreversibly/significantly to the Strep•Tactin matrix.