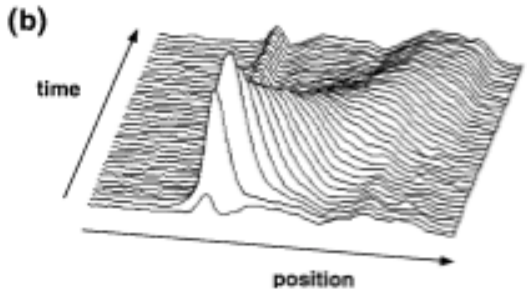
StrepII-tag Purification

The low affinity of the strepII-StrepTactin interaction (k_d~1 μM) causes migration of the adsorption profile down the column (see figure below) in situations of low or zero concentration, as found in dilute samples and during the wash step. *Lengthening the column by connecting 2-3 columns in series and lowering the wash volume prevents the target protein from eluting off the column.*



Ind. Eng. Chem. Res. **2002,** *41,* 2320-2329

Chromatographic parameters	
Column	StrepTrap HP 5mL, 3 in series
Binding Buffer	100mM Tris 150mM NaCl 1mM EDTA pH 8.0
Elution Buffer	100mM Tris 150mM NaCl 1mM EDTA 2.5mM desthiobiotin pH 8.0
Gradient	0-2.5mM desthiobiotin step
Sample Loading Volume	As low as possible (ideally < 100 mL)
Sample Loading Flow Rate	2.5 mL/min
Flow Rate	2.5 mL/min
Wash Volume	1.5 CV (22.5 mL)

1. *Sample preparation.* Concentrate the clarified supernatant 10-20x and buffer exchange into TBS pH 7.4 using tangential flow filtration. The sample volume should be as low as possible. The buffer exchange should be done using at least a 1:10 dilution and then reconcentrated. Residual biotin from the 293 media (Freestyle 293 contains 98 μg/L) binds irreversibly to the StrepTactin ligand which will interfere with binding of the target protein and can inactivate the column. (Addition of avidin was found to be unnecessary.)
2. *Column Equilibration.* Equilibrate the column with 5 CV binding buffer, or until baseline is reached in UV and conductivity. Note: All buffers and samples should be 0.45 μM filtered and degassed.
3. *Sample Loading.* Load the sample onto the column at 2.5 mL/min. For large sample volumes, load half of the sample onto the 3 columns in series, then disconnect the 1st column (entrance). Continue loading onto columns 2 and 3 and then reconnect the 1st column. Collect and save the flow through and save a small volume of the load for SDS-PAGE analysis.

4. *Wash.* After sample application, wash out unbound sample with 1.5 CV (22.5 mL) of binding buffer
5. *Elution.* Elute the strepII-tagged protein by competitive elution using a 2.5mM desthiobiotin step gradient for 2 CV (30 mL).
6. *SDS-PAGE analysis.* Run a gel on the load, flow through, and eluate to check for recovery and purity.
7. *Regeneration.* Remove the bound desthiobiotin by using the following regeneration procedure:
    - 3 CV water
    - 3 CV 0.5M NaOH
    - 3 CV water
8. Repeat steps 2-7 on the flow through of the first purification if SDS-PAGE indicates there is still significant protein remaining in the flow through.

The 5 mL columns should be rotated after each use to prevent a rise in backpressure on the first column. The top column should be moved to the bottom (i.e. from 1-2-3 to 2-3-1 to 3-1-2 to 1-2-3).

Checking the integrity of the column:
HABA provides a visual check of column integrity. HABA undergoes a change in color from yellow to red upon complex formation with StrepTactin. If the upper zone does not turn red, this indicates the column has become inactivated.
- 15 CV Binding Buffer + 1mM HABA
- 30 CV Binding Buffer

Cleaning-in-place (CIP) procedure
If there is an increase in backpressure that does not lower during regeneration, the following cleaning procedure can be used:

- 3 CV water
- 3 CV 0.5M NaOH (removes precipitated proteins)
- 3 CV water
- 3 CV 20% EtOH (removes hydrophobically bound proteins)
- 3 CV water

If the nature of the contaminant(s) is known, the appropriate chemical can be used for CIP as long as it is compatible. The compatability guide can be found at http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/FDE4EB35510A8A6CC1257628001D5EAD/$file/28913630AB.pdf .

References
Schmidt, T.G., Skerra, A. The Strep-tag system for one-step purification and high-affinity detection or capturing of proteins. *Nat. Protocols* **2**, 1528-1535 (2007)

B. Cass et al., Purification of recombinant proteins from mammalian cell culture using a generic double-affinity chromatography scheme. *Protein Expr. Purif.* 40 77–85 (2005)