Cytiva™ Protein Select™ resin

AFFINITY CHROMATOGRAPHY

Cytiva™ Protein Select™ resin is an affinity chromatography resin for purifying recombinant proteins using the self-cleaving Cytiva Protein Select tag.

During an affinity step performed with Cytiva Protein Select resin, the protein self-cleaves from the tag and elutes with no residual tag amino acids.

This technology simplifies tagged protein purification when used in research. It standardizes purification during process development and manufacturing of any protein that does not have an affinity binding partner.

Key features and benefits:

- Simple affinity purification protocol for recombinant proteins.
- One-step purification and tag cleavage without the need for proteases or other elution reagents.
- Delivers a highly pure, native protein with no remaining tag amino acids after tag cleavage.
- Can be processed under mild conditions, which means no exposure to conditions that cause protein aggregation or loss of biological activity.
- Flexible, enabling the use in multiple expression systems.



Fig 1. Cytiva Protein Select resin is available in containers of various volumes and in HiTrap™ prepacked columns.

Recombinant proteins: a family of very diverse molecules

The family of recombinant proteins includes very different types of molecules, such as enzymes, protein scaffolds, hormones, growth factors, etc. They often don't have an affinity binding partner to facilitate their purification. To be able to standardize and simplify the purification with an affinity step, one strategy has been to add a tag to the recombinant protein. Historically, using a tag has mainly been used for research purposes and not for biotherapeutic purification because the tags could not be removed without leaving traces on the target molecules. With Cytiva Protein Select technology, you can now use a tag purification protocol with confidence that the protein will be automatically removed from the tag and eluted with no residual tag amino acids.

Using Cytiva Protein Select resin

A simple purification protocol

Purifying a protein tagged with Cytiva Protein Select tag is simple.

One chromatography step does the affinity purification, a traceless tag cleavage, and the tag removal. The pure protein obtained is in its native state with no traces of the tag amino acids.

The protocol does not require the use of a protease, and the same buffer can be used for equilibration, binding, wash, and elution.

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Principle

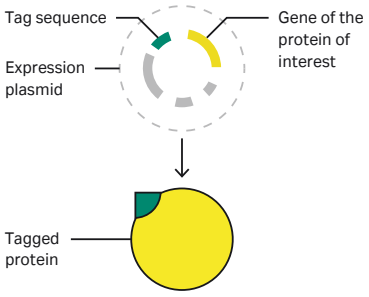
Figure 2 describes how a recombinant protein is purified using Cytiva Protein Select resin and tag, from incorporating the tag to obtaining a pure protein.

1. Protein design and expression

Incorporate the Cytiva Protein Select tag sequence and the gene of your protein of interest into your expression plasmid.

Express the protein in the suitable expression system.

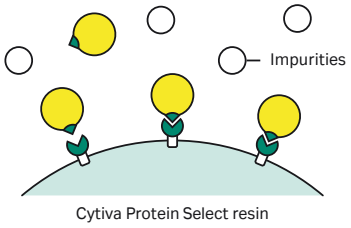
Collect the sample that contains the expressed protein.



2. Binding and wash

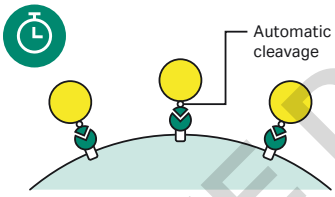
Load your sample onto the Cytiva Protein Select resin.

Your tagged protein binds to the ligand while impurities flow through during the wash.



3. Hold step

Pause the flow and initiate a hold step. Automatic cleavage happens when the ligand and the tag fold together.



4. Elution

Elute your pure, tag-free protein with no residual tag amino acids.

You do not have to change the buffer conditions, but you can if needed.

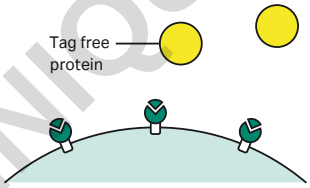


Fig 2. Overview of how Cytiva Protein Select technology works.

Cytiva Protein Select tag

Tag characteristics

Cytiva Protein Select tag is a 36-amino acid tag that has a specific affinity to Cytiva Protein Select resin. The resin and the tag constitute the self-cleaving Cytiva Protein Select technology.

To produce your protein with Cytiva Protein Select tag:

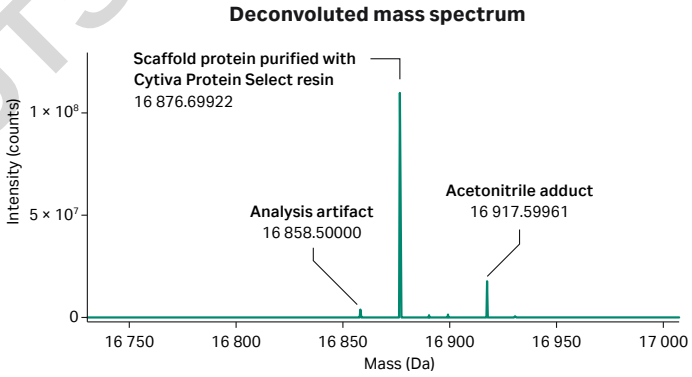
- Get the tag sequence for free by registering at cytiva.com/Protein-Select
- Design your construct so that the tag is located on the N-terminus of your target protein.

Automatic cleavage site

The cleavage site is located between the last amino acid of the tag and the first amino acid of the target protein.

A completely traceless tag cleavage

After self-cleavage, Cytiva Protein Select tag is completely removed with no remaining amino acids left on the protein of interest. Figure 3 shows mass analysis of a scaffold protein purified using Cytiva Protein Select resin and tag. It shows that the mass of the protein obtained exactly equals the expected mass after cleavage, confirming that the protein is the pure, native state with no traces of the tag amino acids.



Protein	Mass
Protein with tag	22 141 Da
Expected mass after tag removal	16 876 Da
Mass measured after purification with Cytiva Protein Select resin	16 876.7 Da

Fig 3. Mass analysis shows that the mass of the protein matches the mass expected after tag cleavage. Mass error = 40 ppm. LC-MS analysis on Waters ACQUITY system using a C8 RPC column.

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Cytiva Protein Select resin

Resin characteristics

Cytiva Protein Select resin is based on the established high-flow agarose matrix, which gives excellent pressure/flow properties. The rigid matrix allows high-flow velocities in recombinant protein purification processes.

Affinity ligand

The ligand that is immobilized to the resin base matrix has a specific affinity to Cytiva Protein Select tag.

Table 1. Characteristics of Cytiva Protein Select resin

Matrix	Highly cross-linked agarose
Particle size, d50V[1]	~ 60 µm
Ligand	Protein ligand derived from *E. coli*
Coupling chemistry	Epoxy
Yield[2]	Up to 20 mg protein/mL resin
Chemical stability and compatibility	Stable in commonly used aqueous buffers
pH stability	Operational: 2 to 10 Regeneration/CIP: see Table 3
Recommended flow velocity[3]	Recommended: 450–600 cm/h Maximum: 600 cm/h
Storage	2°C to 8°C, 20% ethanol
Delivery conditions	20% ethanol

[1] Median particle size of the cumulative volume distribution.
[2] Yield will depend on protein design and cleavage-time (hold time). Yield is the amount of cleaved target protein in the eluate. Since the cleavage process starts when the tagged protein is bound to the resin, it is not possible to measure the traditional dynamic binding capacity (DBC, Q_{B10}) using UV signal.
[3] At room temperature and bed height maximum of 10 cm.

Cytiva Protein Select technology can be used throughout the entire biotherapeutics development process

From early research and drug discovery to process development and GMP manufacturing, this technology facilitates a smoother progress of molecules along the development process.

Simplify purification and tag removal

When working with tagged protein purification during your research or drug discovery work, there are times when leaving the tag on the protein after purification can be acceptable. However, if the tag alters the conformation of the protein or prevents the protein from being crystallized, the tag should be removed to lower the risk of affecting the protein function and to gain a better understanding of the protein's structure.

When working with traditional tags such as His-tag or GST-tag, tag removal is a lengthy process, and some amino acids from the tag remain after tag removal (Fig 4). The remaining amino acids can potentially cause issues with functional and structural studies and impact your results.

One simple step with Cytiva Protein Select resin replaces three steps (the affinity capture step, a tag cleavage, and the tag removal) of a traditional tagged protein purification protocol (Fig 4) without using a protease. Purification of a tagged protein and tag cleavage with Cytiva Protein Select resin can be done in less than one day. Purification with other tags can take several days.

The protocol makes purification simpler, faster, and leaves no unwanted amino acids. The obtained protein will be your protein, and just your protein. As a result, your functional and structural studies will be more reliable.

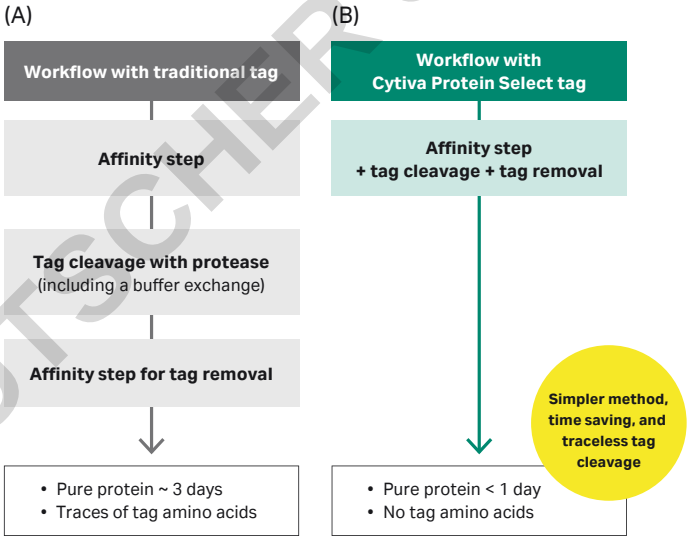


Fig 4. Comparing tagged protein purification workflows (number of steps, time, and presence of tag amino acids traces). (A) Protocol using a traditional tag. (B) Protocol using Cytiva Protein Select tag.

A platform process with traceless tag affinity purification to accelerate process development

When a protein has an affinity binding partner, the purification process can include an affinity capture step, which usually provides high purity level (~ 95%). One or two polishing steps are often sufficient to obtain the final expected purity. However, if a protein does not have an existing affinity binding partner, which is often the case for recombinant proteins, the first chromatography step will be less efficient. As a result, more polishing steps are usually needed, which requires time and effort during process development.

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Adding Cytiva Protein Select tag to the recombinant protein enables the use of an affinity step with Cytiva Protein Select resin thereby standardizing and simplifying protein purification to obtain sufficient purity (Fig 5).

Process development gets easier, faster, more efficient. Fewer chromatography steps are required, which improves process time and cost.

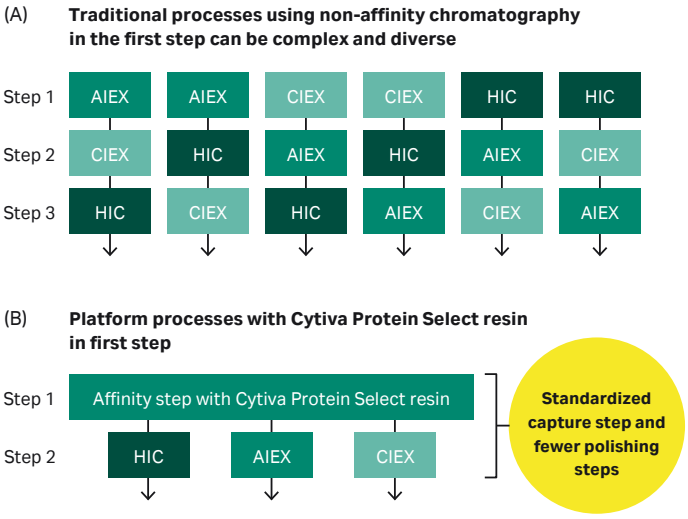


Fig 5. Comparing processes using non-affinity method in first step (A) vs a method using Cytiva Protein Select resin (B) in first step. AIEX = anion exchange chromatography, CIEX = cation exchange chromatography, HIC = hydrophobic interaction chromatography.

Practical considerations

Selection of the protein expression system

There are several different types of expression systems you can use for production of your recombinant protein. Cytiva Protein Select technology is flexible and can be used for different expression systems, secreted and non-secreted.

Proteins tagged with Cytiva Protein Select tag have been expressed in mammalian (HEK and CHO) and *E. coli* expression systems and demonstrated no impact on expression levels of the proteins.

Cleavage characteristics

The cleavage rate is mainly affected by the first 1–3 amino acids of the protein, in particular:

- Hydrophobic and aromatic amino acids in the second position will increase the rate of cleavage.
- Small and nonpolar, or negatively charged amino acids in the first position will slow the rate of cleavage.
- Proline amino acids located in first and/or second position will halt the cleavage. We suggest that you perform a point mutation of the proline amino acid(s).

Furthermore, cleavage may be affected by conditions such as temperature. It is usually slower at lower temperatures.

Hold step time

Once your sample is loaded on to the column and a wash step is operated, a hold step (by pausing the flow) is performed during which the cleavage takes place.

- A 4 hour hold step is recommended for cleavage, but an overnight hold step can be performed.
- The procedure can be performed either at room temperatures or in a cold room.
- The cleavage rate is protein dependent.

Buffer conditions

When using the Cytiva Protein Select resin, your protein can be processed under mild conditions, so it isn't exposed to conditions that cause aggregation or loss of biological activity. A single buffer can be used throughout the entire purification for equilibration, binding, wash, and elution.

Suggested buffers

- MES (pH 6.0 to 6.5)
- Phosphate (pH 6.5 to 7.5)
- Tris (pH 7.5 to 9.0)

It is recommended to use 20 to 50 mM of buffer compound supplemented with up to 1 M salt (e.g., NaCl), at a pH range of 6 to 9.

Resin stability and compatibility

Cytiva Protein Select resin is compatible with commonly used aqueous buffers and a range of additives (Table 2). The resin is stable for one week in these solutions without adverse side effect on its chromatographic performance. Some of the additives are not suitable to use during purification.

Table 2. Buffers and additives that are compatible with Cytiva Protein Select resin

Chemical	Concentration tested
MES	30 mM
HEPES (HBS-N)	10 mM HEPES, 150 mM NaCl, pH 7.4
Phosphate buffered saline (PBS)	10 mM sodium phosphate, 140 mM NaCl, pH 7.4
Tris	50 mM
NaCl	1 M
Sodium dodecyl sulfate (SDS)	0.10%
Dithiothreitol (DTT)	10 mM
β-mercaptoethanol (BME)	40 mM
TCEP	10 mM
Tween 20	1%
EDTA	1 mM
DMSO	10%
Urea	4 M
Acetic acid	0.5 M
Isopropanol	30%
Ethanol	20%
Guanidine hydrochloride	4 M

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Short term exposure (e.g., regeneration and cleaning-in-place (CIP))

For short-term exposure, such as for regeneration and CIP, the resin is stable in NaOH in combination with urea, guanidine hydrochloride (GdnHCl), or isopropanol (IPA). See Table 3 for recommended concentrations.

Table 3. Recommended concentrations for regeneration and CIP solutions

Solution	Acceptable concentration
Urea with NaOH	4 M urea, 100 mM NaOH 6 M urea, 50 mM NaOH
GdnHCl with NaOH	4 M GdnHCl, 100 mM NaOH
IPA with NaOH	30 % IPA, 100 mM NaOH

Resin compatibility during purification

Our testing has shown that Cytiva Protein Select resin is compatible with many common buffers, salts, and excipients, including EDTA, DTT, and divalent metal ions, during purification (Table 4).

Table 4. Substances and concentrations tested in buffers or samples during purification with Cytiva Protein Select resin*

Tested substance	Tested concentration	Comment
Urea	1 M	High concentrations of denaturing substances should be avoided during purification.
Imidazole	250 mM	
EDTA	10 mM	
DTT	2 mM	Other reducing agents may also be compatible
n-decyl-β-D-maltoside (DM)	0.2%	Other non-ionic detergents may also be compatible
Lauryldimethylamine oxide (LDAO)	0.2%	Other zwitterionic detergents may also be compatible; note that use of LDAO has coincided with some decrease in target protein yield

*This list does not preclude other concentrations or similar agents

Regeneration and cleaning-in-place studies

Multiple regenerations of Cytiva Protein Select resin are possible. After each purification, the resin requires regeneration and cleaning-in-place (CIP) to remove the tag, uncleaved protein, and impurities.

We investigated reproducibility in terms of regeneration and CIP by repeated cycles of purification using *E. coli* homogenate or HEK feed containing proteins tagged with Cytiva Protein Select tag. We performed several regeneration and CIP studies, and the conditions we tested are described in Table 5.

Table 5. Conditions of the regeneration and CIP studies performed

Number of cycles tested	20 or 50 cycles
Regeneration and CIP	• Performed in every cycle • Contact time: 15 min • Various solutions tested
Purification cycle conditions	• HiTrap Protein Select column 1 mL • Single buffer • Cleavage time of 3 or 4 h
Sample	• *E. coli* homogenate containing scaffold protein • *E. coli* homogenate containing streptokinase C • HEK293 containing interleukin-1β (IL-1β)

The efficiency of the regeneration and CIP solution varies for different protein samples. Concentration of NaOH and chaotropic agent are typically the main parameters to vary for optimization of the regeneration and CIP. Harsh conditions might, however, lead to a decrease in the retained yield. The nature of the feed material will ultimately determine the optimal regeneration and CIP solution. The results of these studies are presented below and summarized in Table 6.

Table 6. Summary of regeneration and CIP study results

	***E. coli* homogenate containing a scaffold protein** (see Fig 6)	***E. coli* homogenate containing streptokinase C** (see Fig 7)		**HEK293 feed containing IL-1β** (see Fig 8)	
Regeneration and CIP solution	4 M urea, 100 mM NaOH	4 M GdnHCl, 100 mM NaOH	6 M urea, 50 mM NaOH	4 M GdnHCl, 100 mM NaOH	30% IPA, 100 mM NaOH
Retained yield (% of initial protein yield)*	80% (after 20 cycles)	85% (after 20 cycles) 67% (after 50 cycles)	84% (after 20 cycles)	94% (after 20 cycles)	87% (after 20 cycles)
Protein purity*	> 90%	> 90%	> 90%	> 99%	> 99%

*All samples were analyzed by analytical SEC, except for samples with *E. coli* homogenate containing a scaffold protein, where SDS-PAGE was analyzed using ImageQuant™ TL 10.1 software.

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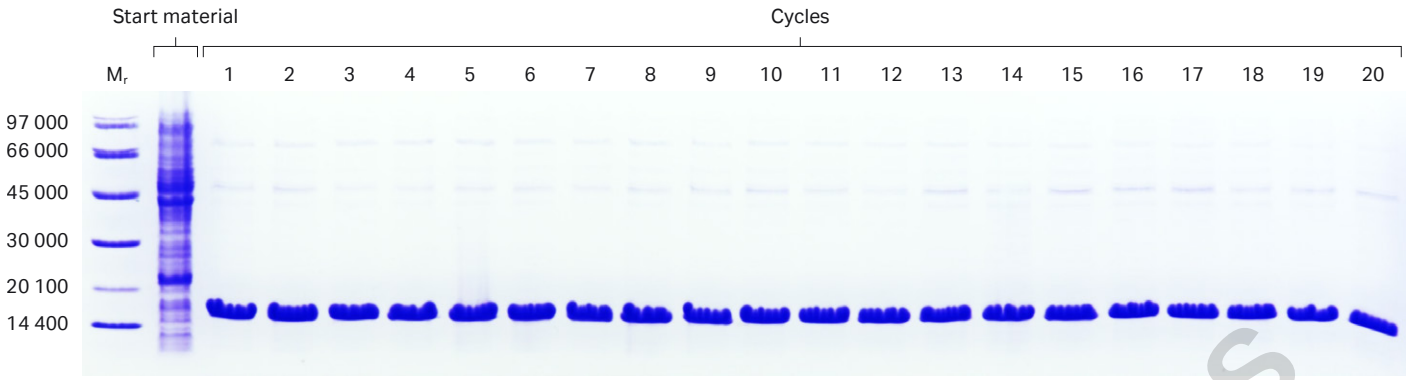


Fig 6. SDS-PAGE analysis (reduced conditions, Coomassie blue stained) of eluted scaffold protein (from *E. coli* homogenate) over 20 purification cycles using a regeneration and CIP solution of 4 M urea, 100 mM NaOH.

Results for scaffold protein from E. coli *homogenate*

Figure 6 shows SDS-PAGE results for each of the 20 purification cycles, using a regeneration and CIP solution of 4 M urea, 100 mM NaOH. A quantitative analysis of the SDS-PAGE using ImageQuant TL 10.1 analysis software showed that after 20 cycles, 80% of the initial yield was retained. The purity of eluted target protein remained stable > 90% in each purification cycle.

Results for streptokinase C from E. coli *homogenate*

Figure 7 shows size exclusion chromatography (SEC) analysis of streptokinase C yield from *E. coli* homogenate over multiple purification cycles using 4 M GdnHCl, 100 mM NaOH (50 cycles) or 6 M urea, 50 mM NaOH (20 cycles) for regeneration and CIP. After 20 cycles, the retained yield was 85% when we used 4 M GdnHCl, 100 mM NaOH, and 84% when we used 6 M urea, 50 mM NaOH. We continued the study with the 4 M GdnHCl, 100 mM NaOH solution for an additional 30 cycles. After a total of 50 purification cycles with 4 M GdnHCl, 100 mM NaOH, 67% of the initial yield was retained. Purity remained stable at > 90% for all cycles (data not shown).

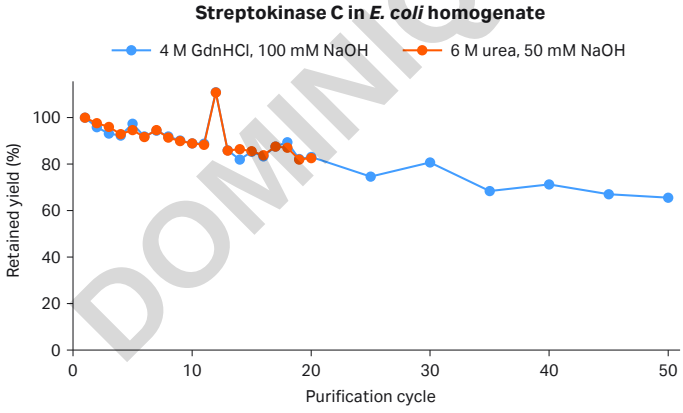


Fig 7. Retained yield for IL-1β expressed in *E. coli*, using different CIP and regeneration solutions. Cycle 12 is an outlier due to an instrument error that increased the cleavage time.

Results from HEK293 feed containing IL-1β

Figure 8 shows retained yield (A) and purity (B) of IL-1β in a HEK293 feed over 20 purification cycles. Retained yield, shown as a percentage of initial yield, was 94% when we used 4 M GdnHCl, 100 mM NaOH for regeneration and CIP, and 87% when we used 30% IPA, 100 mM NaOH. The purity was stable and remained high (> 99%) in all purifications.

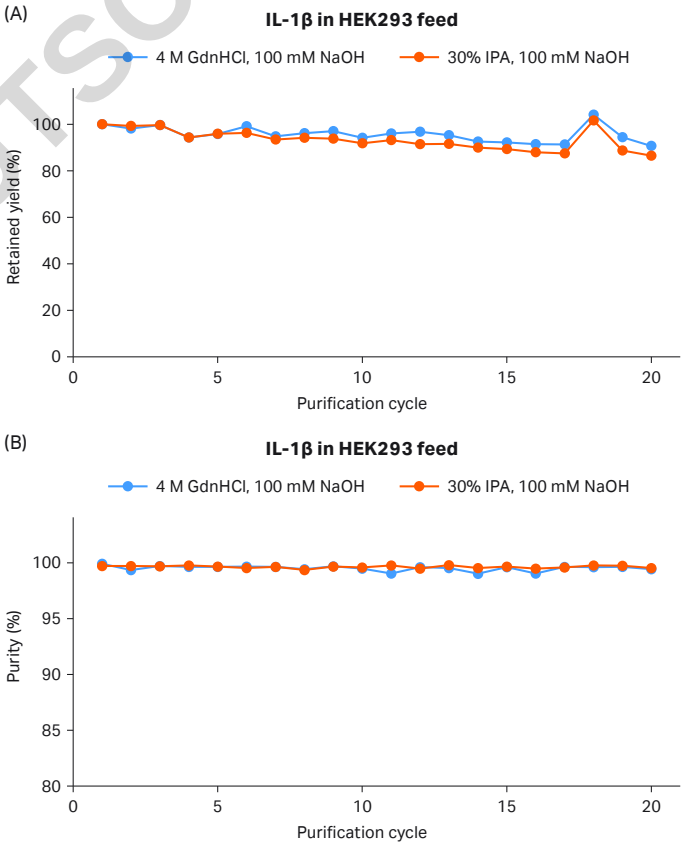


Fig 8. Retained yield in the eluate (Cycle 18 is an outlier due to an instrument error) (A) and purity according to analytical size exclusion chromatography (B) for IL-1β in HEK293 feed, using different CIP and regeneration solutions.

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Applications

One-step purification of the receptor-binding domain (RBD) of SARS-CoV-2 spike protein

Cytiva Protein Select resin was used to purify RBD expressed in HEK293 cells. The purification was done in a single affinity step with a single buffer, and the tag was inserted on the N-terminus. A purity of 97% was achieved with no remaining amino acids left from the tag (Fig 9).

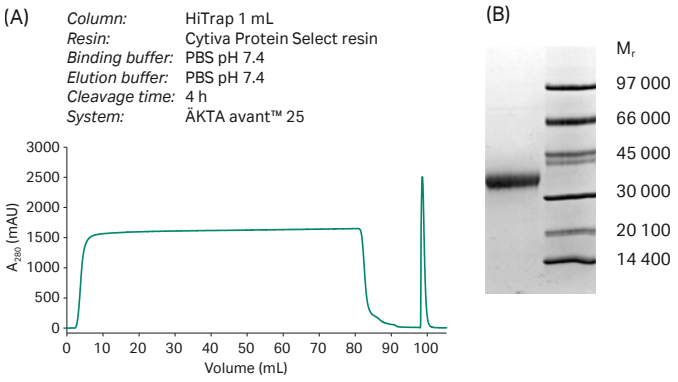


Fig 9. Chromatogram of RBD purification on HiTrap Protein Select column 1 mL with a yield of 10 mg of protein. The corresponding SDS-PAGE shows a purity of 97%.

Comparison with a His-tagged protein purification protocol

In this study, we compared two purification protocols:

- Protocol using a Cytiva Protein Select tag:
  - Construct: IL-1β protein tagged with Cytiva Protein Select tag
  - Column: HiTrap Protein Select column 1 mL
- Protocol using a His-tag:
  - Construct: IL-1β protein tagged with a His-tag, TEV-protease cleavage sequence
  - Column: HisTrap™ HP 1 mL

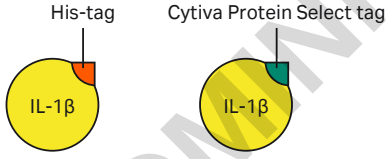


Fig 10. The same IL-1β was tagged with a His-tag or with a Cytiva Protein Select tag. The purification protocols were compared.

We compared the protocol complexity, the time spent, the obtained purity level, and the quality of tag removal. SDS-PAGE analysis (Fig 11) shows that we were able to get a tag-free protein with more than 98% purity, while Figure 12 shows all steps operated to obtain the pure protein and to remove the tag. Table 7 is a summary of the results: the method using a Cytiva Protein Select tag and resin takes less time and less effort compared to a method using a His-tag. Furthermore, the Cytiva Protein Select method did not leave residual tag amino acids on the protein while the His-tag method left tag amino acid traces.

Experimental conditions with Cytiva Protein Select tag methodology

Sample:	*E. coli* lysate containing IL-1β tagged with Cytiva Protein Select tag (IL-1β with a P2F substitution to improve cleavage kinetics – *see* Practical considerations)
Column:	HiTrap Protein Select column 1 mL
Flow rate:	4 mL/min
Cleavage time:	4 h
Buffer:	2 × PBS, pH 7.4
System:	ÄKTA pure™

Experimental conditions with His-tag methodology

***1.** IMAC bind-elute affinity purification of His-tagged IL-1β*

Sample:	*E. coli* lysate containing His-tagged IL-1β
Column:	HisTrap HP 1 mL
Flow rate:	1 mL/min
Binding buffer:	2 × PBS, pH 7.4
Washing buffer:	2 × PBS, 20 mM imidazole
Elution buffer:	2 × PBS, 500 mM imidazole
System:	ÄKTA pure

***2.** Buffer exchange before tag cleavage*

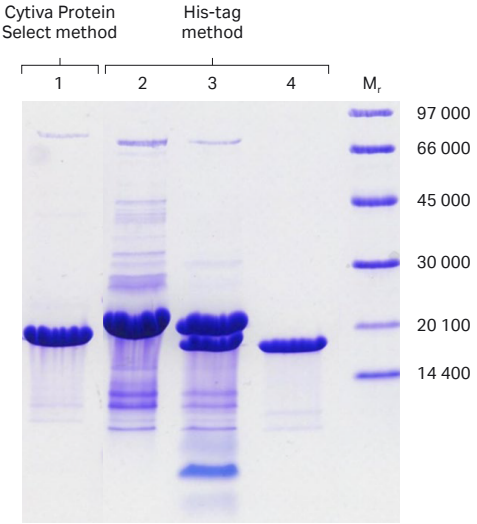
Sample:	Eluted His-tagged IL-1β in IMAC elution buffer: 2 × PBS, 500 mM imidazole
Column:	PD-10 desalting column with Sephadex™ G-25 resin
Sample buffer after buffer exchange:	30 mM Tris, 150 mM NaCl, pH 7.5

***3.** Tag cleavage*

Protease:	His-tagged TEV protease
Tag cleavage:	Sample mixture of His-tagged IL-1β and TEV protease on end-over-end rotation
Tag cleavage buffer:	30 mM Tris, 150 mM NaCl, pH 7.5
Tag cleavage temperature:	Cold room
Tag cleavage time:	Overnight

***4.** IMAC flow-through purification to recover the tag-free protein*

Sample:	Mixture of His-tagged IL-1β and TEV protease after cleavage
Column:	HisTrap HP 1 mL
Sample loading flow rate:	0.5 mL/min
Binding and washing buffer:	30 mM Tris, 150 mM NaCl, pH 7.5
Column washing flow rate:	1 mL/min
Elution buffer:	2 × PBS, 500 mM imidazole
Elution flow rate:	1 mL/min



1. Totally cleaved IL-1β protein from HiTrap Protein Select column 1 mL
2. His-IL-1β purified on HisTrap HP 1 mL followed by desalting
3. TEV-protease cleavage of His-IL-1β
4. IL-1β (flow-through) from HisTrap HP containing residual amino acids

Fig 11. SDS-PAGE analysis shows that both methods result in 98% purity. The method using Cytiva Protein Select technology results in no residual amino acids left on the protein after cleavage unlike the His-tag purification. (LC-MS data not shown).

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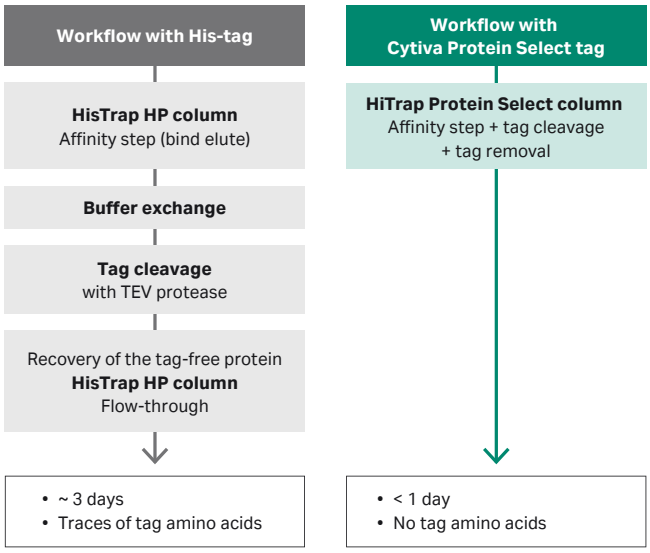


Fig 12. Comparing the protocols using a His-tag and a Cytiva Protein Select tag.

Table 7. Comparison of both methods

	His-tag	Cytiva Protein Select tag
Purity level	98% (contains tag residual amino acids)	98% (no remaining residual tag amino acids)
Time	~ 3 days	< 1 day
Number of steps	4	1
Comparison	• Extra time and effort to cleave and remove the tag • Some tag amino acids stay on the protein after cleavage	• Self cleavage of the tag • Completely traceless tag cleavage

Comparing process development using a non-affinity method vs a method using Cytiva Protein Select resin

As mentioned earlier, the use of tags has not historically been considered when developing processes for biotherapeutic purification, because tags could not be removed without leaving traces on the target molecules. (The costs involved when using proteases is another barrier.) Therefore, it has been very common to use a combination of non-affinity chromatography steps (e.g., HIC + CIEX + AIEX).

In this study, we compared two approaches (Fig 13) for purifying a scaffold protein expressed in *E. coli*. The target protein is a single domain protein (17 kDa) and is thermostable up to 75°C.

We performed the screening phase in small scale (using HiTrap chromatography columns) to identify the techniques and resins to carry forward to larger scale. The requirements were: purity ≥ 95%, recovery ≥ 50%.

The approaches were as follows:

- **Method using non-affinity chromatography steps**, which is typical in process development when purifying a recombinant protein that does not have an affinity binding partner. The approach we chose — AIEX, then heat treatment, then HIC — was the result of an 11-week screening process to define which methodology should be used.
- **Method using Cytiva Protein Select resin** for the capture step, followed by an AIEX polishing step. The screening process for this approach was 3 weeks long.

We transferred the best-performing process from the screening phase to 10 cm bed height columns and compared their performance in term of purity and recovery (see Fig 13).

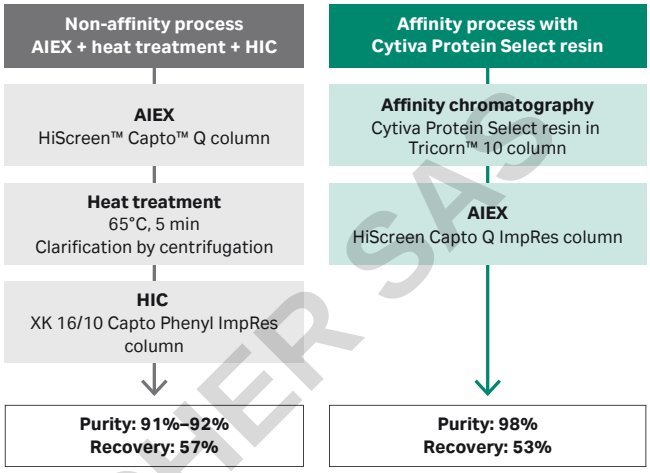


Fig 13. Methods evaluated in parallel on 10 cm bed height columns after the initial resin screening performed on HiTrap columns.

Table 8 is a summary of the comparison (including results from the screening phase and from the final experiments performed with 10 cm bed height columns). Overall, the use of Cytiva Protein Select resin for the first step made it easier and faster to develop a method for reaching a higher level of purity.

Because of the shorter time required for developing this process, a lab could consider increasing the number of candidate molecules going to preclinical studies.

Table 8. Comparison of developed (but non-optimized) methods

Scale	AIEX + heat treatment + HIC	Cytiva Protein Select resin + AIEX
Small scale (HiTrap format)	Purity: 95%	Purity: 98% to 100%
10 cm bed height columns	Purity: 91% to 92% Recovery: 57%, with most of the loss occurring in the first capture step	Purity: 98%, confirmed in analytical SEC, RPC, electrophoresis, and LC-MS analysis Recovery: 53%
Convenience	• Developing a HIC chromatography step required extensive screening and optimization. • The process required the addition of a non-chromatography step (heat treatment). • More stringent pooling criteria or an additional purification step are needed to reach > 95% purity.	• The affinity chromatography does not require optimization. • No need for buffer screening. Only 1 buffer (PBS pH 7.4) needed in affinity step. • 6 h of cleavage.
Development time	Developing the full small-scale process was significantly faster when we used Cytiva Protein Select resin in the first step.	

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Figure 14A shows the results of the non-affinity method described above. The purity obtained was 91% to 92% after transfer to 10 cm bed-height columns (SEC data not shown). The protein would require extra polishing steps to reach higher purity, which would further increase process development time.

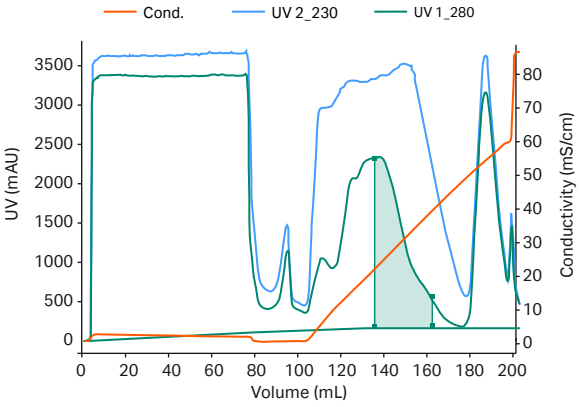
Figure 14B shows the results of the affinity method using Cytiva Protein Select resin. The purity level was > 98% and well above desired target of 95% (SEC data not shown). When performing a mass analysis, the correct mass after tag removal was achieved, showing pure, native protein after cleavage (data not shown).

(A)

Non-affinity process

Step 1: AIEX

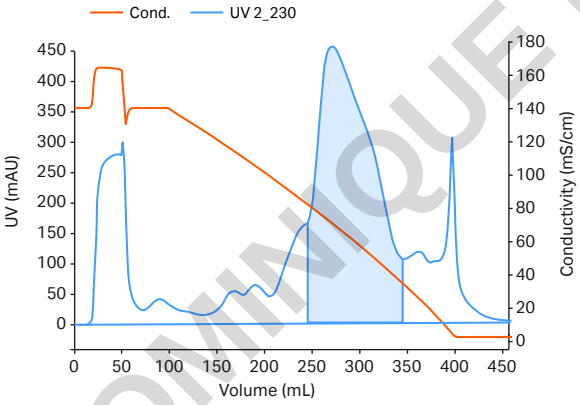
Column: HiScreen Capto Q
Sample load: 47 mg from 12 g *E. coli* cell paste



Step 2: Heat treatment

Step 3: HIC

Column: XK 16/10 Capto Phenyl ImpRes
Sample load: Heat-treated pool from AIEX step

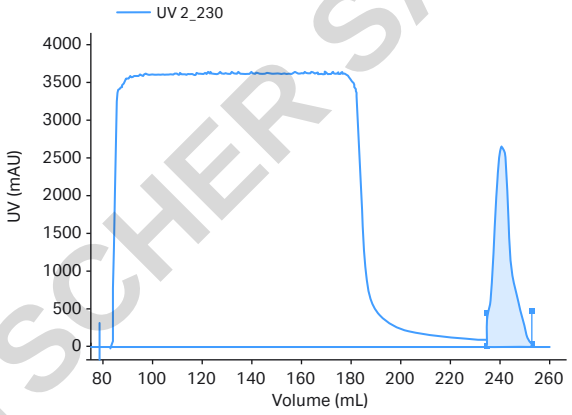


(B)

Affinity process with Cytiva Protein Select resin

Step 1: Cytiva Protein Select resin

Column: Tricorn 10 column packed with Cytiva Protein Select resin, 10 cm
Sample load: 51 mg from 15 g *E. coli* cell paste *(corresponding to 39 mg cleaved)*



Step 2: AIEX

Column: HiScreen Capto Q ImpRes
Sample load: Tagless protein pooled from step 1

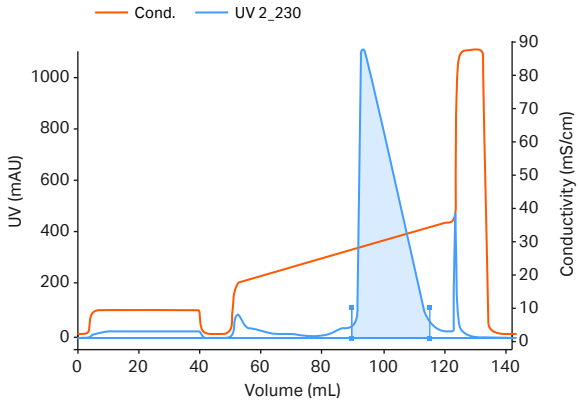


Fig 14. Chromatograms obtained from the non-affinity process (A) and the affinity process with Cytiva Protein Select resin (B). The shaded areas correspond to the pooled fractions.

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A scale-up study of Cytiva Protein Select resin using streptokinase C produced in *E. coli*

Streptokinase C was tagged with Cytiva Protein Select tag and expressed in *E. coli*. The sample was homogenized and purified at two different scales. The columns used were Tricorn 5/100 (10.4 cm bed height) and HiScale™ 26/40 (10.0 cm bed height). Because the resin is more compressed in the larger HiScale column (compression factor 1.31) than in the Tricorn column (compression factor 1.07), we increased the sample volume in proportion to the amount of resin used in column packing (not the column volume). Linear flow velocity was kept constant when scaling up. We determined the parameters (sample load, cleavage time, sample load flow, and temperature) prior to this study.

Columns:	Cytiva Protein Select resin packed in: 1. Tricorn 5/100 (CV 2 mL, 5 mm inner diameter (i.d.), 10.4 cm bed height, compression factor 1.07) 2. HiScale 26/40 (CV 53 mL, 26 mm i.d., 10.0 cm bed height, compression factor 1.31)
Sample:	*E. coli* homogenate containing streptokinase C tagged with Cytiva Protein Select tag
Sample load:	13.1 mL on Tricorn 5 column and 417 mL on HiScale 26 column.
Buffer:	50 mM Tris, 300 mM NaCl, pH 7.5
Flow rate:	400 cm/h during sample load, 600 during wash and 200 cm/h during elution.
Cleavage time:	20 h
System:	ÄKTA avant 25 and ÄKTA pure 150
Purity analysis:	SDS-PAGE and analytical SEC
Yield analysis:	UV 280 nm measurement

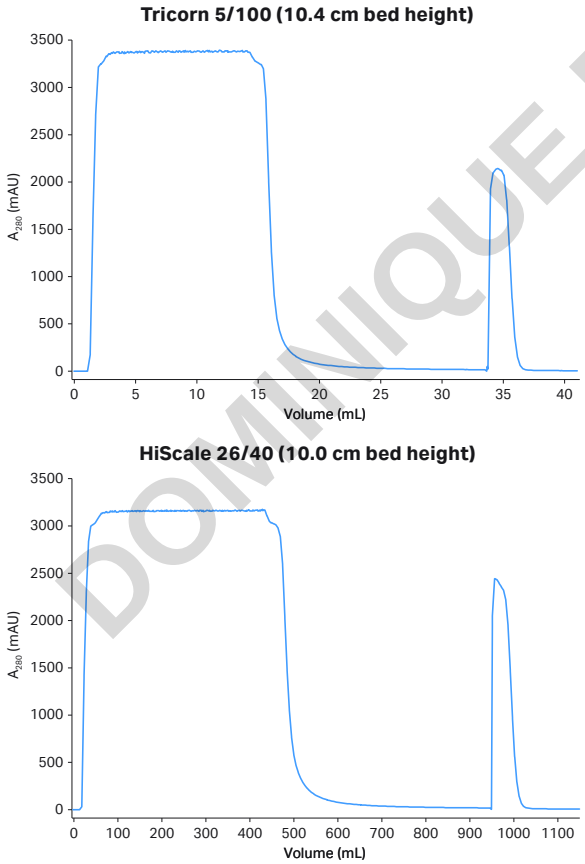


Fig 15. Purification of streptokinase C on Cytiva Protein Select resin packed in Tricorn 5/100 (10.4 cm bed height) and HiScale 26/40 (10.0 cm bed height) columns.

We obtained comparable results using the two column formats with 10 cm bed height: 33.4 mg eluted protein from the Tricorn column purification, and 1034.0 mg from the HiScale column. This corresponds to 15.3 mg and 14.9 mg protein per mL settled resin volume, respectively. Purity was 95% for both column formats (analytical SEC chromatograms not shown).

These data show that chromatographic performance was maintained during scale-up.

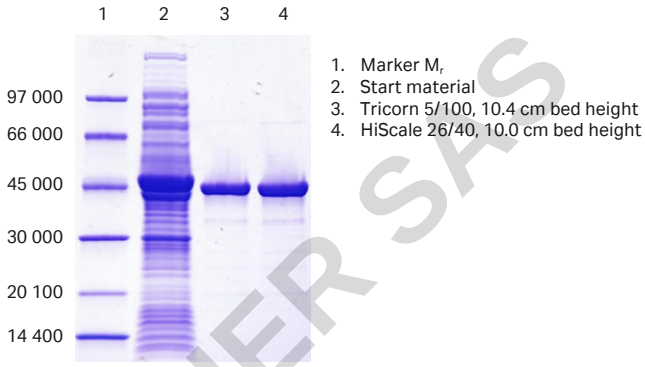


Fig 16. SDS-PAGE analysis (reduced conditions, Coomassie blue stained) showing purification of streptokinase C on Cytiva Protein Select resin packed in Tricorn 5/100 (10.4 cm bed height) and HiScale 26/40 (10.0 cm bed height) columns.

Combining Cytiva Protein Select resin with SEC

Using Cytiva Protein Select resin will allow you to use affinity chromatography to obtain high purity in one step. However, in applications where extremely high purity is required, an additional purification step may be required.

In a study, we purified IL-1β tagged with Cytiva Protein Select tag (Fig 17). The purity obtained after the affinity step using Cytiva Protein Select resin was 96.2%. A total of 6.3 mg protein was collected from the elution.

Column:	HiTrap Protein Select 1 mL
Sample:	*E. coli* lysate containing IL-1β tagged with Cytiva Protein Select tag
System:	ÄKTA pure 25
Buffer:	30 mM Tris, 300 mM NaCl, pH 7.5
Cleavage time:	4 h
Purity analysis:	Analytical SEC and SDS-PAGE

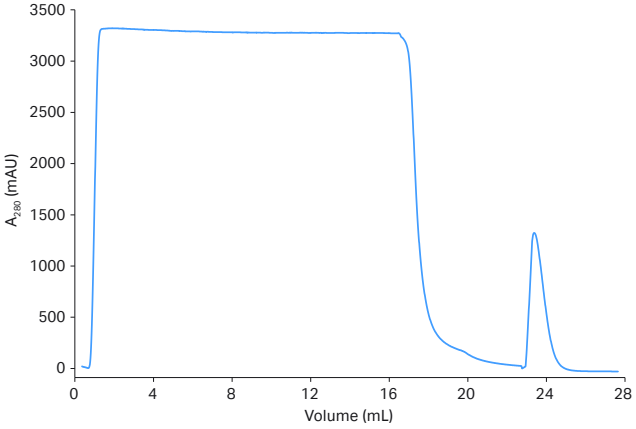


Fig 17. Purification of IL-1β using Cytiva Protein Select resin.

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In this application, a higher level of purity was desired, so a SEC step was added as a polishing step despite the potential loss of yield. We used Superdex™ 75 Increase resin, as it has a molecular weight fractionation range from 3000 to 70 000 Da, which covers most recombinant and small proteins sizes.

As shown in Figure 18, after the sample was run on the Superdex 75 Increase resin packed in a HiScale 16/40 column, the purity obtained was 99.8%. The resulting yield from this run was 5.3 mg of IL-1β.

The purity after the first step using Cytiva Protein Select resin was 96.2%, which is good for many applications. When even higher purity is desired, a SEC step can be added as shown in this case study, where the final purity obtained was 99.8%. The high purity of both steps can be observed in the SDS-PAGE shown in Figure 19.

Sample: Cleaved IL-1β purified with Cytiva Protein Select resin
Column: HiScale 16/40 Superdex 75 Increase
System: ÄKTA pure 25
Buffer: 30 mM Tris, 300 mM NaCl, pH 7.5
Purity analysis: Analytical SEC and SDS-PAGE

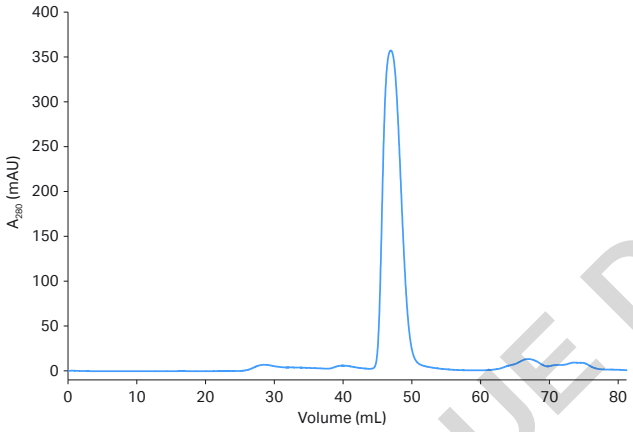


Fig 18. Purity of 99.8% was obtained after the SEC step.

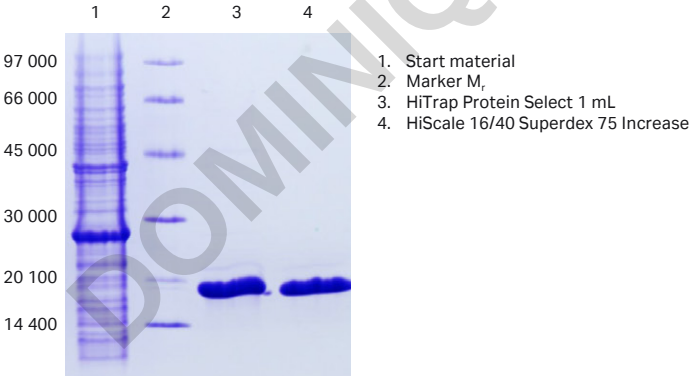


Fig 19. SDS-PAGE of samples after affinity purification and cleavage on HiTrap Protein Select column (lane 3) and after SEC with Superdex 75 Increase resin (lane 4).

Formats for research through to commercial manufacturing

Cytiva Protein Select resin is available in 25 mL, 100 mL, 500 mL, 1 L, and 5 L packs of bulk resins and in HiTrap 1 mL and 5 mL columns, which are well suited for research, process development, and scaling up to commercial scales. Packing methods for Tricorn, HiScale, or AxiChrom™ chromatography columns are available. The resin is shipped in 20% ethanol. We offer other custom prepacked column formats on request, including custom-packed ReadyToProcess™ columns. Contact your Cytiva sales representative for more information.

Regulatory support files (RSF) will be available shortly at cytiva.com/RSF. For a Cytiva Protein Select ligand restricted license, contact your Cytiva sales representative for assistance and updates on availability.

Supply chain stability

The complex nature of biopharmaceuticals makes manufacturing a challenge, in which delivering a consistent, high-quality end product is dependent on the use of equally consistent, high-quality manufacturing components. Cytiva continues to make significant investments in capacity expansion and supply stability to ensure reliable and consistent supply of our chromatography resins. We recommend that you work closely with our commercial teams to forecast demand to support our production planning and manufacturing operations.

For emergency preparedness, we have made significant investments and implemented efforts to minimize the risk and impact of any potential supply interruptions in our manufacturing. Cytiva's chromatography product manufacturing has been certified to ISO22301 Business Continuity Management standards. As an extra precaution in the event of any disruption of our supply chain, we've created a strategic reserve of chromatography resins used in approved manufacturing processes, to support ongoing supply coverage during the recovery phase. Resin types, volumes, and storage locations of the reserve are regularly reviewed to ensure effective deployment of materials globally should an incident occur.

Support

Cytiva Protein Select resin belongs to the BioProcess™ family of products developed and supported for large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term resin supply, and regulatory support files (RSF) to assist process validation and submission to regulatory authorities.

Further reading

- Top challenges in recombinant protein purification process development
- Tag affinity chromatography challenges faced by researchers today
- Frequently asked questions
- eBook: recombinant protein process development with Cytiva Protein Select resin

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Ordering information

Format	Volume	Product code
Bulk resin	25 mL	17542101
	100 mL	17542102
	500 mL	17542103
	1 L	17542110
	5 L	17542104
HiTrap column	1 × 1 mL	17542151
	5 × 1 mL	17542152
	1 × 5 mL	17542153
	5 × 5 mL	17542154

Receive the sequence of Cytiva Protein Select tag for free

Scan this QR code or visit cytiva.com/Protein-Select
The sequence will be sent to you by email today.

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Cytiva Protein Select resin, Cytiva Protein Select tag and use of the same for purification purposes are protected by the following patent applications and foreign equivalents: PCT/EP20/082966, PCT/EP22/062467 and PCT/EP24/061173.

Upon purchase of the Cytiva Protein Select resin from Cytiva and its licensed distributors, customer is granted a free, non-transferable limited license to make and have made biomolecules comprising the Cytiva Protein Select tag, solely for the purpose of purification of said biomolecules using the Cytiva Protein Select resin. Any other use will require a separate license from Cytiva.

The Danaher trademark is a proprietary mark of Danaher Corporation.

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CY38320-16Dec24-DF

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