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 later phases 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 as HiTrap™ prepacked columns and as 25, 100, and 500 mL resin bottles. In the future, larger scale formats will be available.

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.

Principle

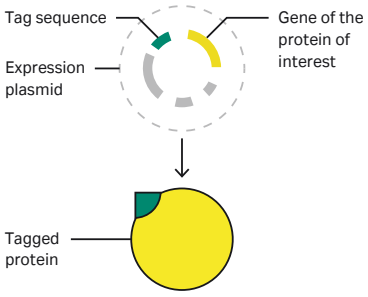
Figure 2 describes how a recombinant protein is purified using Cytiva Protein Select resin and tag, from incorporating the tag to obtaining a pure protein.

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.

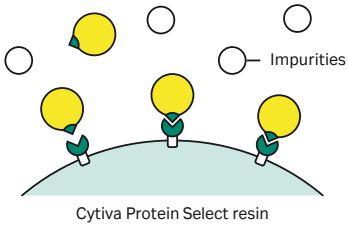
Collect the sample that contains the expressed protein.



2. Binding and wash

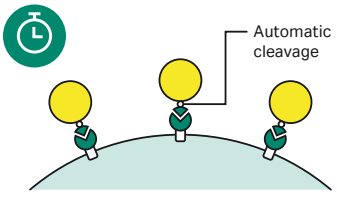
Load your sample onto the Cytiva Protein Select resin.

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.

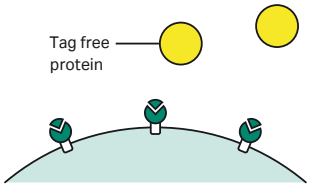


Fig 2. Overview of how Cytiva Protein Select technology works.

Cytiva Protein Select tag

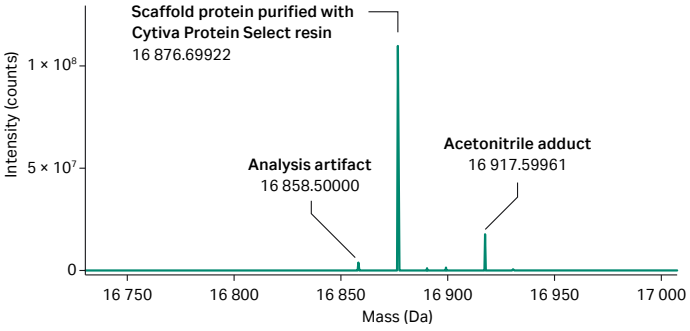
Tag characteristics

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.

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.

Deconvoluted mass spectrum



Protein mass with tag	22 141 Da
Expected protein mass after tag removal	16 876 Da
Obtained protein mass 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.

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.

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.

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: 4 M urea in 0.1 M NaOH
Recommended flow velocity[3]	Recommended: 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.
[3] Maximum bed height: 10 cm

Resin and prepacked formats

Cytiva Protein Select resin is available in 25 mL, 100 mL, and 500 mL packs of bulk resin and in HiTrap 1 mL and 5 mL columns (HiTrap Protein Select columns), which are well suited for research and process development work. Instructions are supplied for packing Cytiva Protein Select resin in Tricorn™ columns.

In the future, support and products for scaling-up to clinical and commercial scale will be available including regulatory support files and ligand leakage kit. Larger resin containers (1 L, 5 L, and 10 L) will eventually be available for packing in larger columns, such as HiScale™ or AxiChrom™ chromatography columns.

For convenience, we will also offer other custom prepacked column formats on request. Contact your Cytiva sales representative for more information.

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

From early research and drug discovery to process development and GMP manufacturing, this new 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.

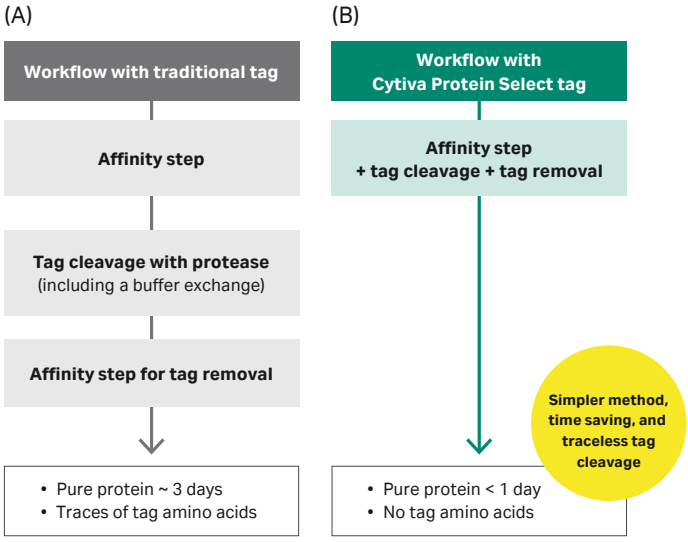


Fig 4. Comparing tagged protein purifications workflows (number of steps, time, and presence of tag amino acids traces). (A) Protocol using a traditional tag. (B) Protocol using Cytiva Protein Select tag.

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.

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.

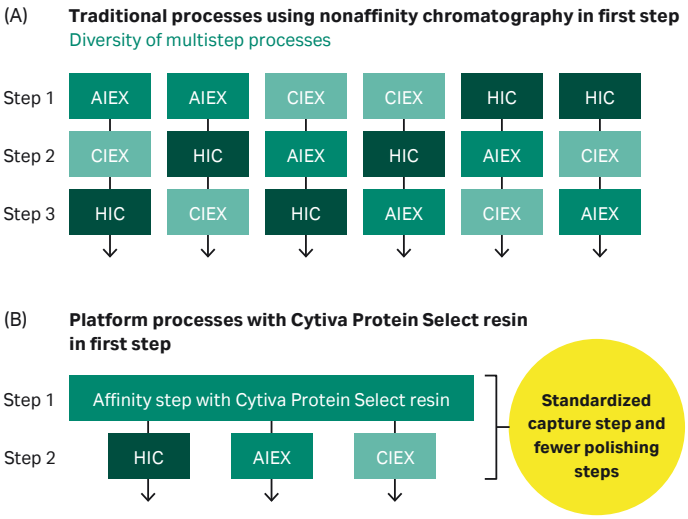


Fig 5. Comparing processes using non affinity method in first step (A) vs a method using Cytiva Protein Select resin (B) in first step. AIEX = anion exchange chromatography, CIEX = cation exchange chromatography, HIC = hydrophobic interaction chromatography.

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 time 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 (preferably 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 in these solutions for one week.

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

Chemical	Concentration
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

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 to NaOH in combination with urea and guanidine hydrochloride. See Table 3 for recommended concentrations.

Table 3. Recommended concentrations for regeneration and CIP solutions

Solution	Acceptable concentration
Urea combined with NaOH	4 M urea, 0.1 M NaOH
Guanidine hydrochloride combined with NaOH	4 M guanidine hydrochloride, 0.1 M NaOH

Regeneration and cleaning-in-place

Multiple regenerations of Cytiva Protein Select resin are possible without losing significant binding capacity. Indeed, after each purification, the resin needs regeneration and cleaning-in-place to remove the tag, uncleaved protein, and impurities.

Reproducibility in terms of regeneration and cleaning-in-place (CIP) was investigated by repeated cycles of purification in HiTrap Protein Select column 1 mL. In the study, the homogenate of a protein tagged with Cytiva Protein Select tag (expressed in *E. coli*) was used, and the cycle runs were performed using a single buffer and a cleavage time of three hours. Regeneration and cleaning-in-place were performed in every cycle using a solution containing 4 M urea and 100 mM NaOH with a contact time of 15 min.

Figure 6 shows the SDS-PAGE results for each the 20 cycles. 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 cycle.

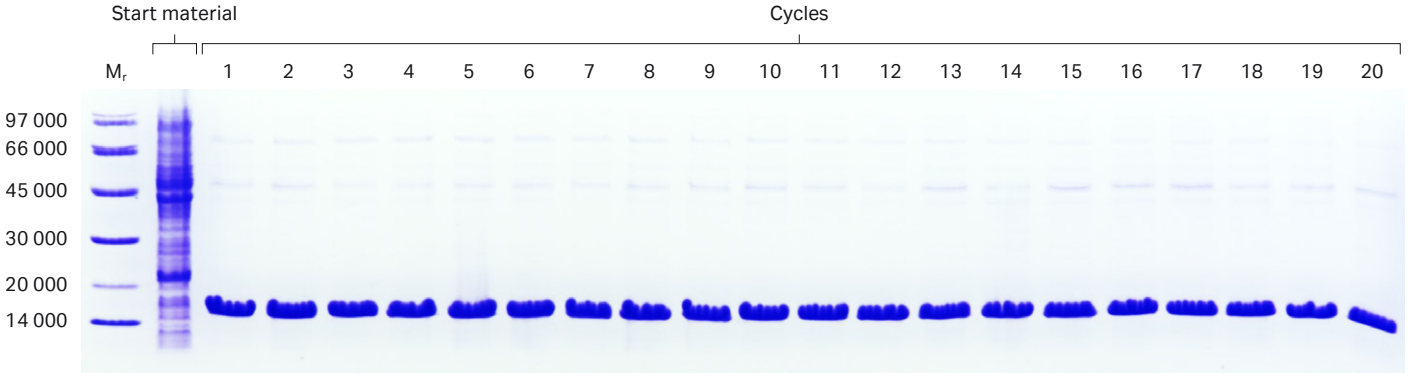


Fig 6. SDS-PAGE analysis showing eluted protein from 20 purification cycles (reduced conditions, Coomassie blue stained).

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 7).

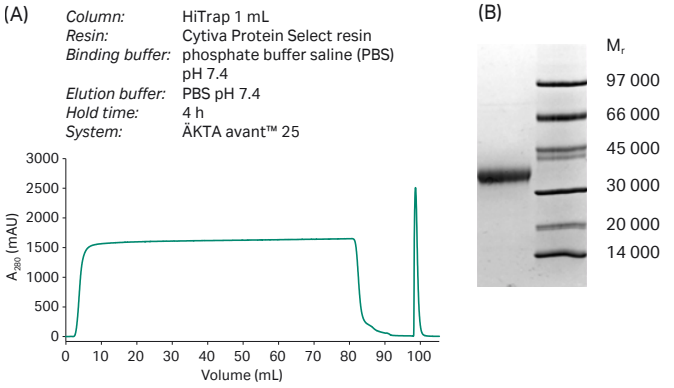


Fig 7. Chromatogram of RBD purification on HiTrap™ Protein Select™ column 1 mL with a yield of 10 mg of protein. The corresponding SDS-Page shows a purity of 97%.

Comparison with a histidine-tagged protein purification protocol

In this study, we compared two purification protocols:

- Protocol using a Cytiva Protein Select tag:
  - Construct: Interleukin-1β (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 histidine tag (His-tag), TEV-protease cleavage sequence
  - Column: HisTrap™ HP 1 mL

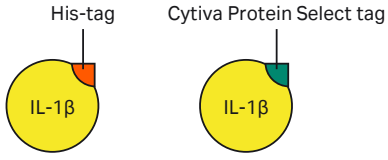


Fig 8. The same IL-1β was tagged with a His-tag and with a Cytiva Protein Select tag. The purification protocols were compared.

We compared the protocol complexity, the time spent, the obtained purity level, and the quality of tag removal. SDS-page analysis (Fig 9) shows that we were able to get a tag-free protein with more than 98% purity, while Figure 10 shows all steps operated to obtain the pure protein and to remove the tag. Table 4 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 histidine tag. Furthermore, the Cytiva Protein Select method did not leave residual tag amino acids on the protein while the histidine tag method left tag amino acid traces.

Experimental conditions with Cytiva Protein Select tag methodology

Sample:	IL-1β (2nd Pro amino acid changed to Phe amino acid to improve cleavage kinetic – *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
Load:	*E. coli* lysate containing IL-1β tagged with Cytiva Protein Select tag
System:	ÄKTA pure™

Experimental conditions with histidine-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*

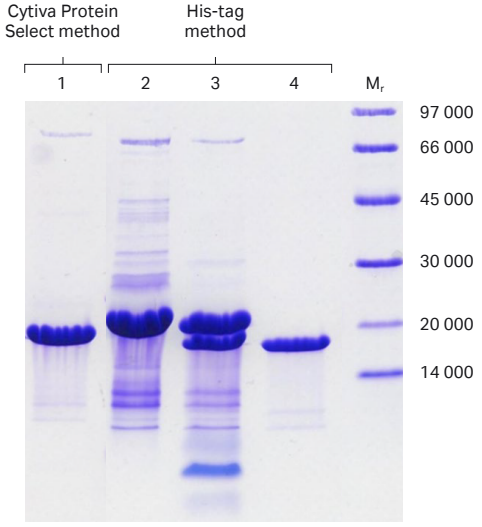
Sample:	Eluted His-tagged IL-1β in IMAC elution buffer: 2 × PBS, 500 mM imidazole
Column:	PD-10 desalting column with Sephadex™ G-25 resin
Sample buffer after buffer exchange:	30 mM Tris, 150 mM NaCl, pH 7.5

***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 9. 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).

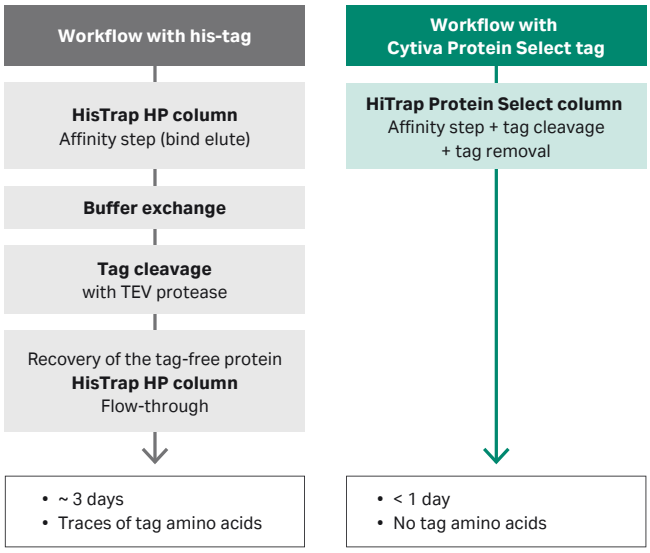


Fig 10. Comparing the protocols using a His-tag and a Cytiva Protein Select tag.

Table 4. 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 nonaffinity method vs a method using Cytiva Protein Select resin

As mentioned earlier, when developing processes for biotherapeutic purification, the use of tags has not been considered so far 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 nonaffinity chromatography steps (e.g., HIC + CIEX + AIEX).

In this study, we compared two approaches (Fig 11) for purifying a scaffold protein expressed in *E. coli*:

- **Method using nonaffinity chromatography steps** when purifying a recombinant protein that does not have an affinity binding partner, which is typical in process development. The choice of using AIEX then heat treatment then HIC was the outcome of a long screening process to define which methodology should be used.
- **Method using Cytiva Protein Select resin** for the capture step.

We compared the complexity and time spent in developing the process.

The target protein is a single domain protein (17 kDa) and is thermostable (up to 75°C).

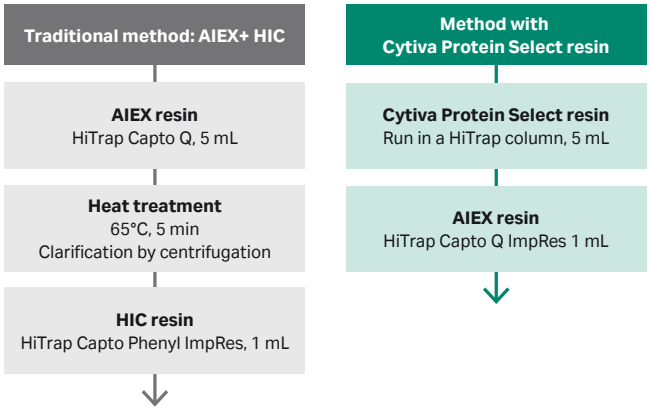


Fig 11. Methods evaluated in parallel.

Table 5 is a summary of the comparison. 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, the lab can consider increasing the number of candidate molecules going to preclinical studies.

Table 5. Comparison of both methods

	AIEX + HIC	Cytiva Protein Select resin + AIEX
Convenience	• Developing a HIC chromatography step required extensive screening and optimization. • Required the addition of a non-chromatography step (heat treatment).	• This affinity chromatography does not require optimization. • No need for buffer screening.
Time spent for developing the process	Developing the full process was two times faster when Cytiva Protein Select resin was used in the first step	
Purity level	95%	Close to 100%

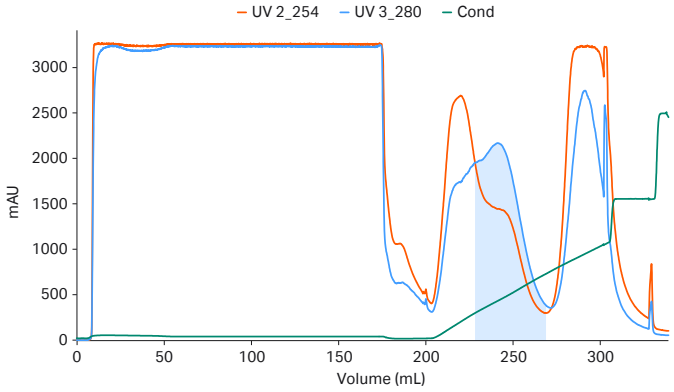
Figure 12 shows the results of the method using AIEX as a first chromatography step followed by heat treatment and then by HIC as second chromatography step. The purity level obtained was 95% (which was the goal). The protein would require extra polishing steps to reach higher purity levels, which would further increase process development time.

Figure 13 shows the results of the method using Cytiva Protein Select resin followed by an anion exchange step. The purity level was almost 100%. When performing a mass analysis, the correct mass after tag removal was achieved, showing pure, native protein after cleavage (*see* Figure 3).

Traditional method: AIEX + heat treatment + HIC

Anion exchange chromatography

Column: HiTrap Capto™ Q 5 mL
Flow rate: 5 mL/min
Sample load: 150 mL



Hydrophobic interaction chromatography

Column: HiTrap Capto Phenyl ImpRes 1 mL
Flow rate: 1 mL/min
Sample load: 38 mL

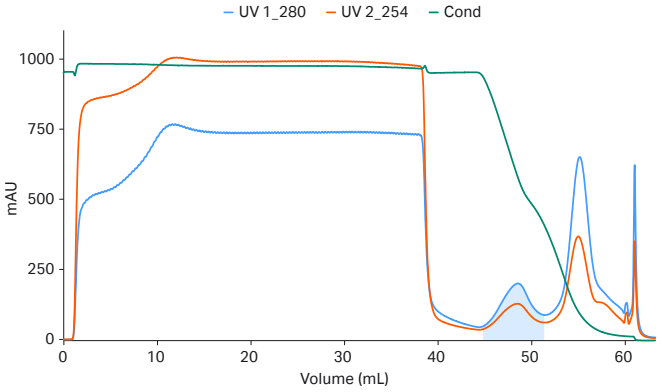
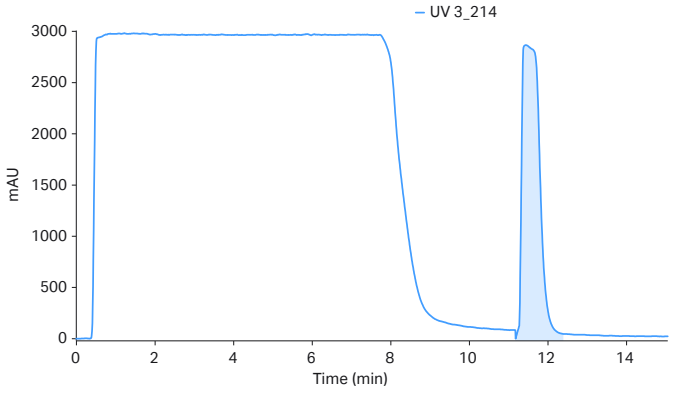


Fig 12. Chromatograms for the AIEX and HIC steps (the blue areas correspond to the fraction containing the target protein). A heat precipitation step was applied between the AIEX and HIC steps. The purity obtained after the HIC step was 95%.

Method with Cytiva Protein Select resin

Affinity chromatography

Column: HiTrap Protein Select column 5 mL
Flow rate: 5 mL/min
Sample load: 40 mL



Anion exchange chromatography

Column: HiTrap Capto Q ImpRes 1 mL
Flow rate: 1 mL/min
Sample load: 4 mL

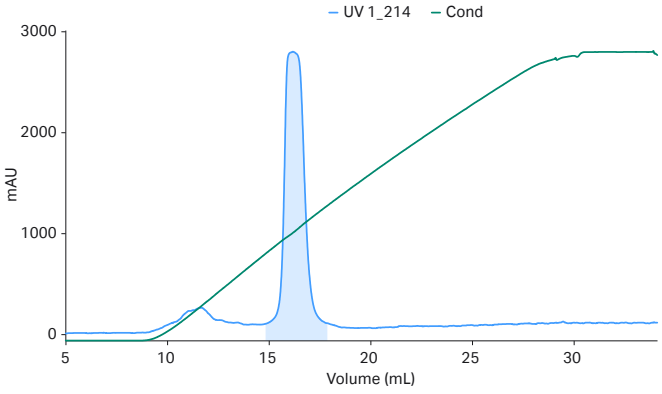


Fig 13. Chromatograms for the affinity step with Cytiva Protein Select resin and the AIEX step (the blue areas correspond to the fraction containing the target protein). The purity obtained after the AIEX step was almost 100%.

Combining Cytiva Protein Select resin with size exclusion chromatography (SEC)

Using Cytiva Protein Select resin will allow you 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 an Interleukin-1β (IL-1β) protein tagged with Cytiva Protein Select tag. The purity obtained after the affinity step using Cytiva Protein Select resin was 96.2%, as shown in Figure 14. A total of 6.3 mg protein was collected from the elution.

Column: HiTrap Protein Select 1 mL
Sample: *E. coli* lysate containing Interlukin-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

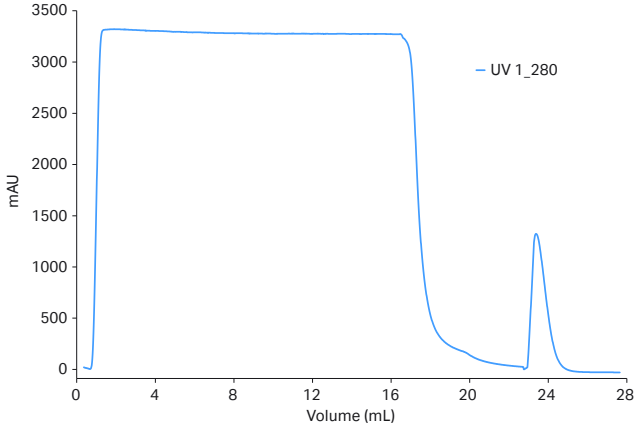


Fig 14. Purification of Interleukin-1β using Cytiva Protein Select resin to obtain a purity of 96.2%.

In this application, a higher level of purity was desired, so a size exclusion chromatography step was added as a polishing step despite the potential loss of yield. Superdex™ 75 Increase resin was chosen due to having a molecular weight fractionation range from 3000 to 70 000 Da that covers most recombinant and small proteins sizes.

As shown in Figure 15, after the sample was run in 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 Interleukin-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 size exclusion chromatography 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 16.

Sample: Cleaved Interleukin-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

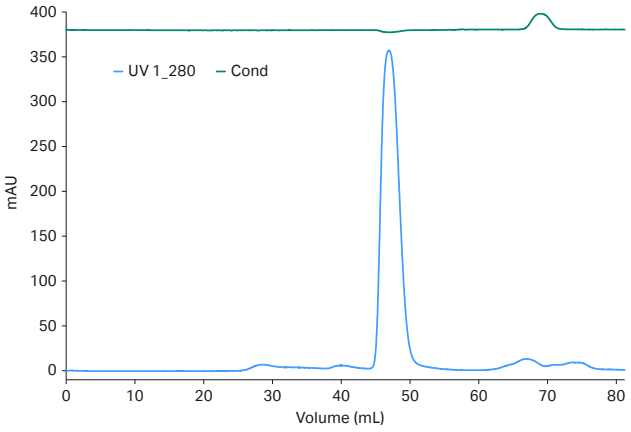


Fig 15. Purity of 99.8% was obtained after the SEC step.

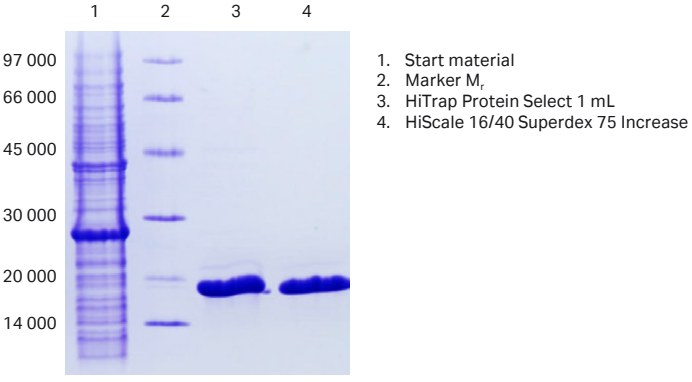


Fig 16. SDS-PAGE of samples after affinity purification and cleavage on HiTrap Protein Select column (lane 3) and after SEC with Superdex 75 Increase resin (lane 4).

Further reading

- Top challenges in recombinant protein purification process development
- Tag affinity chromatography challenges faced by researchers today

Ordering information

Format	Volume	Product code
Bulk resin	25 mL	17542101
	100 mL	17542102
	500 mL	17542103
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

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