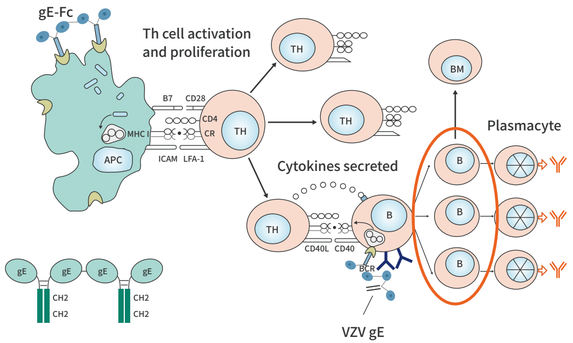

Biological reorganization of herpes zoster vaccine, use of Protein SupAt Beads for purification

Fc-fusion protein	Construct	Expression system	Ligand and Function	Therapeutic use	FDA approval and company
Etanercept (Enbrel®)	TNFR2: hIgG1 Fc	CHO	Inhibit the binding of TNFα or TNFβ to TNF receptor	Rheumatoid arthritis	1998, Amgen/Pfizer
Alefacept (Amevive®)	LFA3: hIgG1 Fc	CHO	Binds to CD2 on T cells,and inhibits the crosstalk of APCs and T-cells thereby inhibiting T-cell activation	Psoriasis and transplant rejection	2003, AstellasPharma
Abatacept (Orencia®)	Mutated CTLA-4: hIgG1 Fc	CHO	Binds to CD80 and CD86 on APCs and thereby inhibits CD28 mediated co-stimulation of T-cells	Rheumatoid arthritis	2005, Bristol/Myers Squib
Romiplostim(NPlate®)	TPO-binding peptide: hIgG1 Fc	E. coli	Acts as an agonist onTPO receptor to stimulate production of platelets in refractory immune thrombocytopenia	immune thrombocytopenia	2008, Amgen/ Pfizer
Rilonacept (Arcalyst®)	IL-1R: hIgG1 Fc	CHO	Binds and neutralizes IL-1	Cryopyrin-associated periodic syndromes	2008, Regeneron/ Sanofi Aventis
Belatacept (Nulojix®)	CTLA-4: hIgG1 Fc	CHO	Binds to CD80 and CD86 and inhibits CD28 mediated co-stimulation of T-cells	Transplant rejection	2011,Bristol/Myers Squib
Aflibercept (Eylea®)	VEGFR1/VEGFR2: hIgG1 Fc	CHO	An antiangiogenic fusion protein designed to bind VEGF-A, VEGF-B and PlGF, all of which are implicated in tumor angiogenesis	Wet age-related macular degeneration	2011,Regeneron/Sanofi Aventis
Ziva flibercept (Zaltrap®)	VEGFR1/VEGFR2: hIgG1 Fc	CHO	An antiangiogenic fusion protein designed to bind VEGF-A, VEGF-B and PlGF all of which are implicated in tumor angiogenesis	Colorectal cancer	2012, Regeneron/ vSanofi Aventis
Eloctate	FVIII: hIgG1 Fc	HEK293	Blood clotting factor	Hemophilia A	2014, Biogen Idec
Alprolix	FIX: hIgG1 Fc	HEK293	Blood clotting factor	Hemophilia B	2014, Biogen Idec

Fc-tagged protein drugs get approved by FDA

Pre-Activated Medium:

Many high-value proteins, particularly therapeutic biologics, often lack commercially available affinity medium due to nonspecific binding sites or the inability to tag fusion proteins. When standard medium are ineffective or yield low purification efficiency, activation medium can be considered.

The first step involves preparing antibodies or heavy chain variable regions (single-domain antibodies) against the target protein. These materials, typically used as excipients, face relatively relaxed regulatory requirements. After the process development and optimization, load determination, stability verification, and resin life verification, they can be approved for commercial production.

Pre-Activated Medium

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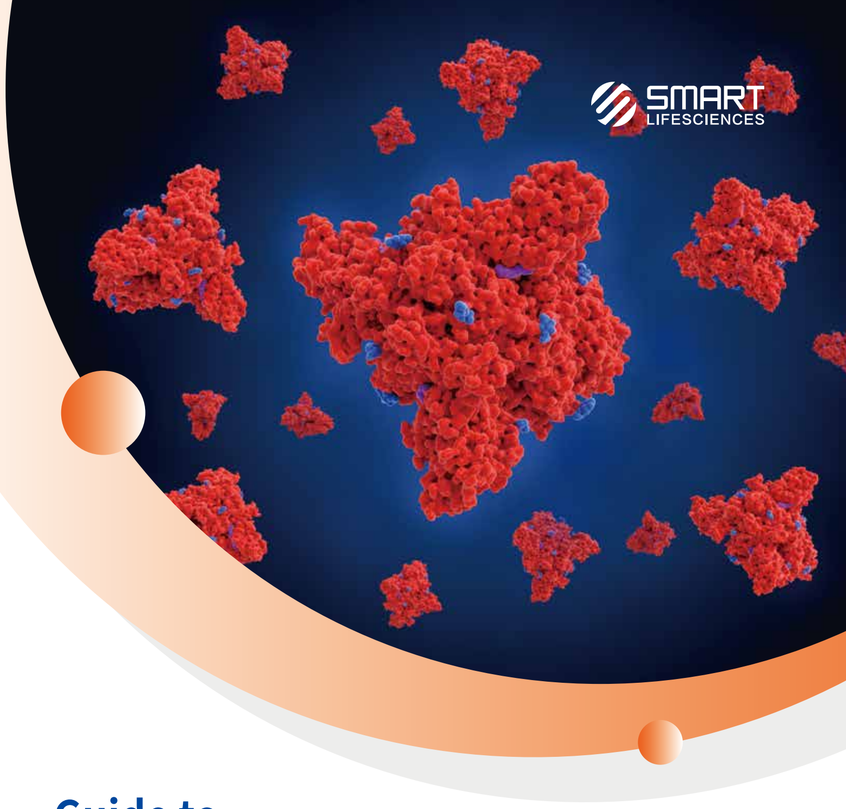


SMART LIFESCIENCES

Guide to Recombinant Protein Purification

Professional Manufacturer
of Chromatography Resin

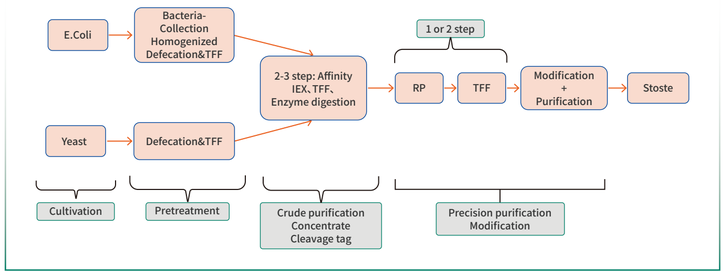
Recombinant protein

Recombinant proteins are highly important biological products, especially in the production of hormones and cytokines, disease prevention and control, and basic research. Depending on actual needs, recombinant proteins can be classified into two types: labeled and unlabeled. Consequently, their purification processes differ significantly.

Non-tag recombinant protein :

Non-tagged recombinant proteins are usually expressed in E. coli, yeast, mammalian cells, insect cells, and plant cells. Before the purification process, it is necessary to confirm the titers, location, and structure of the target protein. According to the specific situation, appropriate treatment methods are selected to enhance the efficiency and recovery rate of the target product.

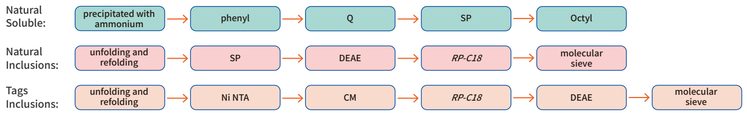
Taking insulin purification as an example, in the E. coli expression system, recombinant insulin exists in the form of inclusion bodies, thus requiring collection after homogenization. After denaturation, renaturation, ion exchange, enzyme digestion, and ultrafiltration, crude insulin can be obtainezd. Drug substances can be obtained after 1-2 steps of reverse phase chromatography (Fig. 2) (Some insulin types may also require side chain modification and additional purification steps).



Classical Insulin purification process

Limited by factors such as cost control, experimental conditions, and culture medium sources, various methods have been developed to extract high-purity proteins. For example, in the case of human interferon, there are at least three process routes: natural soluble, natural inclusions, and tagged inclusions (Fig.2).

The soluble expressed product is more active but contains more impurities, making crude purification relatively difficult. For inclusion bodies, most host cell proteins (HCP) and host cell debris (HCD) can be removed during the pretreatment stage. However, this process is not factory-friendly, particularly during the unfolding and refolding phases, which are characterized by large volumes and lower yields. Considering the misfolding issues, additional purification steps may be necessary.



There are significant differences in the purification methods of recombinant human interferon rhINFa-2b under culture conditions

Generally, the purification process for non-tagged proteins is diverse. Therefore, ion exchange chromatography (IEX) and hydrophobic interaction chromatography (HIC) are commonly used resins. If these resins do not meet the requirements, reversed phase chromatography can be further considered.

Tag fusinon recombinant protein :

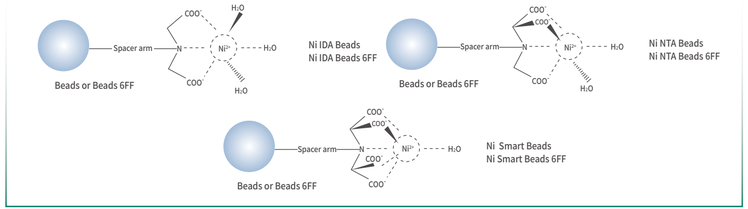
Tags in recombinant proteins are very common as they not only increase purification efficiency but also improve expression and solubility, addressing many questions associated with challenging proteins. There are various types of fusion protein tags, including GST-tag, Flag-tag, RFP/GFP-tag, MBP-tag, Biotin-tag, Strep-tag, and His-tag, with His-tag and GST-tag being the most frequently used.

His-tag:

The His-tag consists of 3-10 consecutive histidine residues, with the 6×His-tag being the most widely used. It can form stable coordination bonds with metal ions such as $Ni^{2+}$and Co^{2+}, allowing impurities to flow through or be eluted with other buffers, thus purifying the target protein.

The His-tag has several characteristics: simple ligand, high capacity, mild separation conditions, versatility, and little impact on protein structure/activity. In most cases, tag removal is unnecessary, making it a powerful tool for recombinant protein purification. It can purify histidine-tagged proteins from various expression sources.

Commonly used His-tag resins include Ni IDA Beads 6FF, Ni NTA Beads 6FF, and Ni Smart Beads 6FF. Purification can be performed under conventional or denaturing conditions.



Schematic representation of the structure of Ni IDA,Ni NTA and Ni Smart

Ni Smart Beads 6FF exhibit a strong binding affinity for Ni2+, enabling efficient purification of target proteins even in the presence of specific concentrations of EDTA and DTT without the need for buffer exchange or concentration steps. They can be directly used to capture and purify His-tagged proteins from the supernatant of eukaryotic cell cultures.

Chemical stability of Ni Smart Beads and Ni Smart Beads 6FF

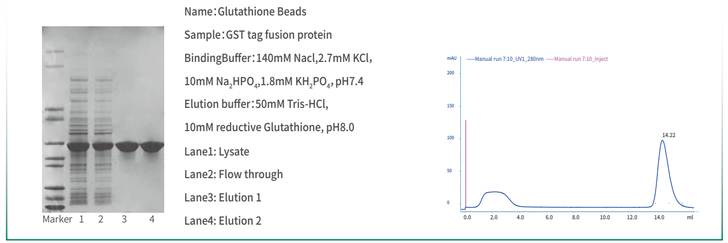
Reagent	Tested time
0.01 M HCl,0.01 M NaOH	One week
10 mM EDTA, 1 M NaOH, 5 mM DTT, 5 mM TCEP,20 mM Mercaptoethanol, 6M Guanidine	24 hours
500mM imidazole,100mM EDTA	2 hours
30% Isopropanol	20 min

GST-tag:

Glutathione S-transferase (GST) is a macromolecular protein with a molecular mass of approximately 26 kDa. The specific binding of glutathione (GSH) to GST can be leveraged to produce affinity resin by coupling GSH' s SH-groups to agarose and other materials.

GST-tagged proteins exhibit specific complementary binding to the glutathione ligand, and the target protein can be eluted by adding reduced glutathione to the buffer. During the binding process, impurities are removed by flow-through or elution with the buffer to achieve separation.

If the GST tag needs to be removed after purification, enzyme digestion or on-column cleavage can be employed.



SDS-PAGE analysis of purification of GST fusion protein

Fc-tag:

The Fc-tag, derived from the constant fragment (Fc domain) of an IgG antibody, is a novel affinity tag that enhances the detection, purification, and localization of proteins. It also positively impacts protein yield, solubility, stability, folding, and activity. The Fc-tag is widely used in drug discovery, drug delivery, vaccine design, and research on receptor-ligand interactions.

In general, the Fc-tag has the following advantages for fusion proteins:

1. Dimerization and multimerization;
2. Improved flexibility;
3. Increase the serum half-life;
4. Improve the stability and solubility of recombinant proteins;
5. Increase expression or secretion of recombinant proteins;
6. Easy detection of Fc-tagged recombinant proteins;
7. Strong specificity for affinity chromatography with protein A or G;
8. Activation and inhibition of signal transduction;
9. Activation or inhibition of cytokine secretion.