# Protein Purification

Protein purification, the process of isolating specific target proteins from tissues, cells, or protein mixtures through extraction and purification techniques, yields proteins of high purity, activity, and yield. Effective purification reduces the impact of impurities on experimental results and ensures the consistency and reliability of biological products.

**Sample Pre-treatment**
· Lysis · Centrifugation · Filtration

**Sample Purification**
· Rough purification
· Medium purification
· Fine purification

**Sample Post-treatment**
· Desalting · Buffer exchange

## 1.Sample Pre-treatment

For intracellular proteins, selecting an appropriate sample pretreatment method is critical to effectively disrupt cell walls and membranes, thereby releasing the target proteins. Common cell lysis techniques include enzymatic digestion, ultrasonic disruption, and mechanical shearing. Table 1 summarizes four types of lysis buffers, allowing users to choose the most suitable option based on experimental needs.

After lysis, the mixture of cell debris and protein solution is typically subjected to centrifugation to separate the supernatant from the pellet. For secreted proteins, centrifugation is also required to remove cells and collect the supernatant containing the protein of interest. However, the resulting supernatant may still contain small molecules and particulate impurities that can affect downstream purification. At this stage, further clarification through filtration—such as microfiltration, ultrafiltration, or nanofiltration—is recommended.

***Table 1: Comparison of Lysis Buffer***

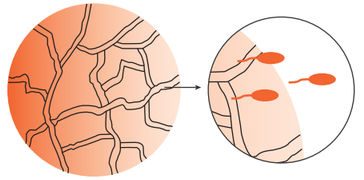
| Product Name | TieChui E.coli Lysis Buffer | RIPA Lysis Buffer (Strong,Contains Inhibitors) | Universal Lysis Buffer | Cut E.coli Lysis Kit |
|---|---|---|---|---|
| Catalog Number | BR0005 | BR0123 | BR0127 | SLR01401 |
| Effective Lysis Components | Lysozyme 、Non-ionicDetergent | Non-ionic Detergent、Anionic Detergent | Non-ionic Detergent、Anionic Detergent | Non-ionic Detergent |
| Lysis Intensity | Mild | Strong | Mild | Mild |
| E.coli Bacteria Lysis | Very Good | Good | Fairly Good | Very Good |
| Mammalian Cell Lysis | Good | Very Good | Fairly Good | Good |
| Membrane Protein Extraction | NO | Very Good | Good | NO |
| Nuclear Protein Extraction | NO | Very Good | Good | NO |
| Compatibility with Different Sample Types | Targeted E. coli | Very Good | Very Good | Targeted E. coli |
| Lysis Time | 1-10min | 1-10min | 15min | 5-10min |
| Contains Nuclease | Yes | NO | NO | Yes |
| ContainsNuclease | NO | Yes | Yes | NO |
| ContainsPhosphatase Inhibitors | NO | NO | Yes | NO |
| Main Applications | Protein purification | WB、IP | WB、Protein purification、IP、COIP、pull down | Protein purification |

## 2.Sample Purification

Protein purification generally consists of three steps: Capture, Intermediate Purification, and Polishing . Currently, Boyi has a complete purification product line, offering hundreds of resin and magnetic bead products. You can select appropriate purification products based on factors such as the properties of the protein to be purified and downstream application requirements (For detailed product information, please refer to the Chromatography Packing Selection Guide by Smart-Lifesciences).

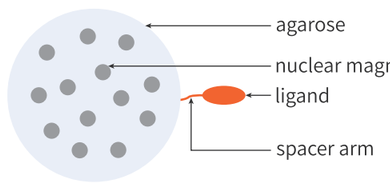
### 2.1 Chromatography Resins

#### 2.1.1 Select appropriate microsphere materials according to experimental conditions

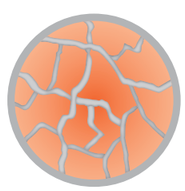


**Agarose microspheres:** Using cross-linked agarose as the matrix, they possess excellent physical and chromatographic properties, can handle large-volume samples, are easy to operate, and are resistant to conventional working conditions such as temperature, pH, and chemical reagents during the chromatography process.

**Dextran microspheres:** Microspheres formed by cross-linking and polymerizing dextran with cross-linking agents through ether bonds, featuring a uniform mesh structure. Their working principle mainly utilizes the molecular sieve effect of dextran microspheres to separate substances based on differences in their molecular sizes.

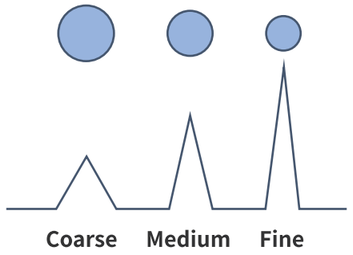



**Magnetic agarose microspheres:** Agarose is coated on the surface of magnetic cores to form superparamagnetic agarose microspheres. These microspheres eliminate the need for expensive chromatography equipment, making the binding and separation of samples with magnetic beads extremely simple and rapid. Additionally, they facilitate the realization of high-throughput and automated protein purification methods more easily.



**Polymer microspheres:** Using polymers as the matrix, which is surface hydrophilically modified and then bonded with ion exchange groups. They possess both good compatibility with large bioactive molecules and excellent physical and chemical stability, greatly improving purification efficiency. The designed pore sizes are widely used for the purification of large biological molecules such as recombinant proteins, antibodies, enzymes, polysaccharides, nucleic acids, and viruses.

### 2.1.2 Select appropriate microsphere particle sizes according to the purification stage




**Coarse:** Suitable for samples with certain viscosity or large-volume samples

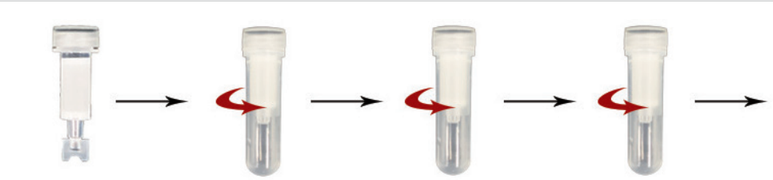
**Medium:** General separation and purification

**Fine:** Fine purification

### 2.1.3 Select appropriate molecular sieve products based on the separation range

***Dextran gel filtration media are mainly used for the separation and purification of polypeptides and proteins, as well as molecular weight determination, etc.***

| Products | Separation Range(globulin) |
|---|---|
| Smartdex G-10 | 700 |
| Smartdex G-15 | 1500 |
| Smartdex G-25 | 1,000-5,000 |
| Smartdex G-50 | 1,000-30,000 |
| Smartdex G-75 | 3,000-70,000 |
| Smartdex G-100 | 4,000-150,000 |




G25 offers desalting columns, pre-assembled columns, and gravity columns for you to choose from!

***Agarose gel filtration media are commonly used for the separation of large proteins, peptides, polysaccharides and the determination of molecular weights***

| Products | Separation Range(globulin) |
|---|---|
| Smartarose 4B | 70,000-20×$10^6$ |
| Smartarose 6B | 10,000-4×$10^6$ |
| Srnartarose CL-4B | 70,000-20×$10^6$ |
| Srartarose CL-6B | 10,000-4×$10^6$ |
| Smartarose 4FF | 40,000-20×$10^6$ |
| Smartarose 6FF | 10,000-4×$10^6$ |

### 2.1.4 Select appropriate hydrophobic media according to hydrophobic properties

| Butyl Beads 4FF | Octyl Beads 4FF | Phenyl Beads 6FF ( Low Sub ) | Phenyl Beads 6FF ( High Sub ) |
|---|---|---|---|

Choose a suitable hydrophobic interaction medium by evaluating the relative hydrophobicity of the target protein versus contaminants:
Under normal circumstances, the hydrophobicity follows this order: Butyl < Octyl < Phenyl, and LS-Phenyl < HS-Phenyl. All these products are available in pre-packed column formats.

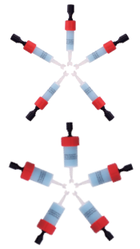
### 2.1.5 Select the appropriate affinity chromatography medium based on the specificity of the ligand

***(1) Tagged Proteins***

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Selectivity** | Ni Smart Beads ⌄ Ni NTA Beads ⌄ Ni IDA Beads | **Purity** | Ni Smart Beads ⌄ Ni NTA Beads ⌄ Ni IDA Beads | **Chemical Resistance** | Ni Smart Beads ⌄ Ni NTA Beads ⌄ Ni IDA Beads | **Binding Capacity** | Ni IDA Beads ⌄ Ni NTA Beads ⌄ Ni Smart Beads |

**Characteristics of Different Metal Ion Ligands**

Metal Ion Binding Affinity: $Cu^{2+}>Ni^{2+}>Zn^{2+}>Co^{2+}$
Target Protein Purity： $Co^{2+}>Zn^{2+}>Ni^{2+}>Cu^{2+}$



Pre-packed columns feature standard interfaces, compatible with various medium-pressure chromatography systems, and can be operated even under manual syringe conditions.
The 6FF series offers higher pressure resistance and flow rates compared to Beads.

IDA ligand resins are more suitable for His-tagged proteins expressed solubly in prokaryotic systems.
NTA ligand resins tolerate moderate concentrations of reducing agents, detergents, denaturants, and chelators, offering broader applicability than IDA.
Smart ligand resins are compatible with higher levels of reducing agents and chelators, enabling direct purification of His-tagged proteins from eukaryotic systems without pre-treatmentSmart ligand resins are compatible with higher levels of reducing agents and chelators, enabling direct purification of His-tagged proteins from eukaryotic systems without pre-treatment.

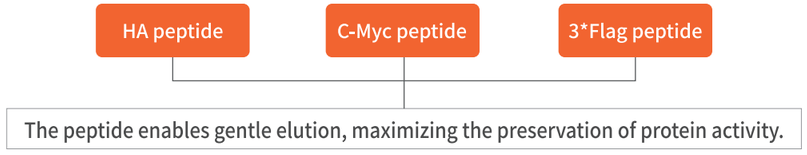
**Need Higher Purity?**

Alternative Metal-Chelating Affinity Resins
Co NTA Beads/6FF
Co Smart Beads 6FF

Cobalt ions exhibit higher specificity for tagged proteins, resulting in purified samples with superior purity

| Tagged Proteins | Products |
|---|---|
| Proteins with an HA-tag | Anti-HA Affinity Beads |
| Proteins with a GFP-tag | Anti- GFP Affinity Beads |
| Proteins with a Flag-tag | Anti-DYKDDDDK Affinity Beads |
| | Anti-DYKDDDDK S1 Affinity Beads |
| Proteins with a c-Myc | Anti-c-Myc Affinity Beads |

**Corresponding peptides according to different labels**




| | |
|---|---|
| Protein with MBP tag | Dextrin Beads /6FF |
| Protein with GST tag | Glutathione Beads/4FF |
| Protein with biotin tag | Streptavidin Beads 6FF |
| Protein with Strep-tag II | Streptavidin ST Beads 4FF、STarm Beads 4FF |
| Protein with Protein A tag | Rabbit IgG Beads 4FF |

The 4FF and 6FF series products are available as pre-packed columns suitable for medium-pressure chromatography systems, and gravity flow columns are also offered as related products.

### *(2) Antibody Products*

| | |
|---|---|
| Human IgG, dog and pig IgG, mouse IgG, rabbit IgG, etc. | rProtein A Beads/ 4FF |
| Human IgG & Livestock Antibodies (e.g., Bovine, Sheep, Horse) | rProtein G Beads/4FF |
| IP (Immunoprecipitation) & Co-IP (Co-Immunoprecipitation) for Antigen-Antibody Detection | rProtein A/G Beads 4FF |
| Human & Mouse Kappa Light Chain Antibody Purification | rProtein L Beads/ 4FF、Smac L |
| Monoclonal Antibody (mAb) Purification | Protein At Beads 4FF、Protein At Beads LX |
| Purification of Target Proteins via Amino Groups on Carrier Proteins | NHS-Activated Beads 4FF、NHS-Activated LA Beads 4FF |
| Purification of Target Proteins via Thiol Groups on Carrier Proteins | PabPur SulfoLink Beads/ 4FF |

### *(3) Untagged proteins*

| | |
|---|---|
| Endotoxin Removal | Endotoxin Removal Beads and kits |
| Glycoproteins, membrane proteins, glycolipids, polysaccharides, membrane vesicles with mannose or glucoside residues, IgM, hormones, lipoprotein purification | Con A Beads 4FF<br>LCA Beads 4FF |
| Purification of albumin, interferon, enzymes requiring nucleotide assistance, 2-macroglobulin, and coagulation factors | Blue Beads 6FF |
| Purification of antithrombin 1, coagulation factor lipoproteins, enzymes, white synthetic nucleic acid conjugating enzymes, restriction endogenous hormones, steroid receptors, cutin, stem cell resistin, etc. | Heparin Beads 6FF |
| Glycoprotein purification | Boric Acid Beads 4FF |
| Serine protease, trypsin, protease-like enzyme purification | Benzamidine Beads 4FF |

## 2.2 Purification Magnetic Beads

Compared to traditional purification resins, magnetic bead-based purification products offer the advantages of lower reagent consumption, higher throughput, and no requirement for expensive instrumentation.

| | |
|---|---|
| Pull-down,His-tag | Ni IDA Magarose Beads |
| | Ni NTA Magarose Beads |
| | Ni Smart Magarose Beads |
| Pull-down，GST-tag | Glutathione Magarose Beads |
| IP, Pull-down,Antibody purification | rProtein A Magarose Beads |
| | rProtein G Magarose Beads |
| IP, Pull-down,flag-tag | Anti-DYKDDDDK Magarose Beads |
| IP, Pull-down,GFP-tag | Anti-GFP Magarose Beads |
| IP, Pull-down,amino coupling | NHS-Activated Magarose Beads |

### *Multi-functional Magnetic Rack*

The multi-functional magnetic rack features an ingenious design compatible with various centrifuge tube sizes including 1.5mL, 2.0mL, 15mL, and 50mL. With its high magnetic field strength, the rack enables rapid and efficient separation, achieving an average magnetic separation time of just 1 minute. The magnetic stand is universally compatible with all magnetic bead products and can be used for sequencing, antibody purification, immunoprecipitation, co-immunoprecipitation, cell sorting, nucleic acid isolation, and other applications.

## 2.3 Others

In addition to the selection of methods and products for protein purification mentioned above, we have also developed kits for exosome and endotoxin removal for you to choose from.

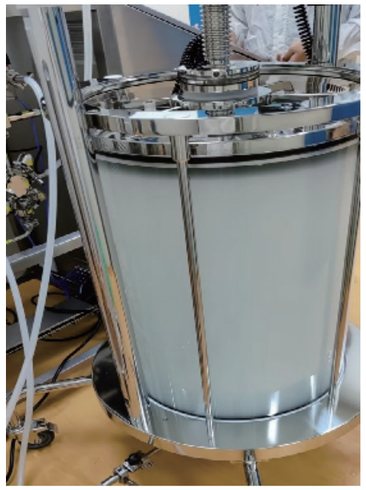
| Product Name | Catalog Number | Advantages | Application |
|---|---|---|---|
| Exosome Rapid Purification Kit | BK0047 | Low cost and simple operation | Want to quickly concentrate and judge whether it contains exosomes or not |
| Exosome Affinity Purification Kit | BK0046 | Low price, low equipment dependence, can quickly capture the target product in a short period of time | Captures a variety of vesicles containing cell membrane structures |
| Exosome Rapid Purification Kit (Column Method) | BK0056 | Simple composition and reusable | Further purification and recovery of highly concentrated exosome samples |
| ENDOCLING® Endotoxin Removal Kit | BK0059 | Magnetic bead method, convenient and quick, low equipment dependence | Endotoxin removal |

# 3.Sample Post-treatment

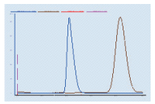
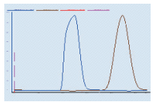
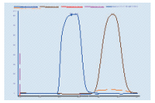
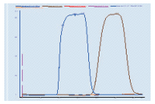
Purified samples prepared in different buffer systems can be transferred into a stable buffer system through dialysis, desalting, or ultrafiltration. For long-term storage, appropriate additives can be added, or samples can be stored under optimized temperature conditions.To meet desalting requirements across different volumes and equipment configurations,we offer a range of desalting column products for your selection.

| Products |  0.5ml SpinDesalt Column |  3ml SpinDesalt Column |  10ml SpinDesalt Column |  P96 SpinDesalt Column |  8.3ml Prepacked Desalting Column |  1ml PreCap Desalting |  5ml PreCap Desalting |  20ml HiPur Desalting |
|---|---|---|---|---|---|---|---|---|
| Required Equipment | Centrifuge | | | | Gravity column rack | Protein purifier | | |
| Loading volume | 100-200 µl | 350 µl-750 µl | 1-2.5 ml | 60-180 µl | 1.0-2.5 ml | 100 µl | 500 µl | 2 ml |
| Time | 5-10 min | 10-15 min | | | 10-20 min | 10-30 min | | |
| Volume change | Unchanged | | | | Dilute 1.5-3 times | Dilute 1.5-2 times | | |
| Application | Fast medesalting of small volume | | Fast desalting of small volume | High throughput fast desalting of small volume | Fast desalting of middle volume | Fast desalting of small volume | | Fast desalting of middle volume |

If you have a large-volume sample requiring desalting, you can choose different sizes of chromatography packing based on the sample volume (we offer column packing services). The separation efficiency between proteins and small-molecule salts depends on the loading volume of the desalting column. Generally, it is recommended that the maximum sample loading volume should not exceed 30% of the column volume.



***Application case***

| | Recovery rate | Resolution | 16/20 |
|---|---|---|---|
| Load 10% | 86% | 4.25 |  |
| Load 20% | 87.1% | 3.68 |  |
| Load 30% | 85.1% | 3.46 |  |
| Load 40% | 89.9% | 0.851 |  |