

## Chelating Agrose (IDA) 6FF

Cat. No.: M1523007 | Pack size: 5ml; 25ml | Storage: Store at 2-8°C, Do not freeze

### Overview

Immobilized Metal Ion Affinity Chromatography separates and purifies proteins based on the specific binding between certain amino acids on the protein surface (e.g., histidine, tryptophan, cysteine) and metal ions ( $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ , etc.).

This product is a metal chelate affinity chromatography medium (without chelated metal ions) prepared by coupling iminodiacetic acid (IDA) ligands to 6% highly cross-linked agarose. It provides three coordination sites for chelating metal ions, and the loaded metal ions further offer three coordination sites for binding proteins, featuring high binding capacity. After the user completes metal ion chelation, the medium can be applied to the separation and purification of proteins and polypeptides in the downstream process of biopharmaceuticals and bioengineering.

Aladdin Chelating Agrose (IDA) 6FF is stored in 20% ethanol, with a gel-to-protectant volume ratio of 7:3. The product specification refers to the actual gel volume.

| Parameter                | Specification                                                                                                                                                                        |
|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Matrix                   | 6% Highly cross-linked agarose                                                                                                                                                       |
| Functional group         | Iminodiacetic Acid (IDA)                                                                                                                                                             |
| Average particle size    | 90 $\mu\text{m}$                                                                                                                                                                     |
| Metal chelation capacity | 30-41 $\mu\text{mol Cu}^{2+}/\text{mL}$ medium                                                                                                                                       |
| Recommended flow rate    | <400 cm/h                                                                                                                                                                            |
| Recommended pressure     | <0.3 MPa (3 bar)                                                                                                                                                                     |
| Working pH               | 4–8.5                                                                                                                                                                                |
| pH stability             | 3–12 (long-term); 2–14 (short-term <sup>①</sup> )                                                                                                                                    |
| Chemical stability       | Stable in common buffers such as 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, etc. Avoid the use of reducing agents and chelating agents such as DTT, EDTA, citrate, etc. |
| Storage conditions       | 20% ethanol; 2–8°C                                                                                                                                                                   |
| Shelf life               | 5 years                                                                                                                                                                              |

**Note:**

① Refers to the stability of the medium without metal ion chelation. Long-term: pH range where stability is maintained without adverse effects on subsequent performance. Short-term: pH range for regeneration, cleaning-in-place and disinfection based on experience.

## Instructions for Use

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### 1. Column Packing

#### 1.1 Preparation of Packing Buffer

Purified water, degas by ultrasonication for 15 min.

#### 1.2 Preparation of Chromatography Medium

Calculate the required amount of chromatography medium (compression coefficient  $\sim 1.15$ ) and weigh for standby. Transfer to packing buffer using a vacuum filtration flask. Add an appropriate amount of packing buffer to the exchanged medium to prepare a  $\sim 50\%$  gel suspension.

#### 1.3 Preparation of Chromatography Column

Inspect the chromatography column to ensure all components are intact and clean. Install the lower end cap, tighten the O- ring, fix the column vertically on a iron stand, and check and adjust with a level to keep the column upright. Draw packing buffer with a syringe, connect to the column outlet, slowly push out the liquid to remove air bubbles from the bottom sieve, remove the syringe and screw on the plug. Add  $\sim 2$  cm height of packing buffer to the column.

#### 1.4 Column Packing (Take a 16 mm diameter, 10 cm bed height column as an example)

Mix the gel suspension evenly, pour slowly into the column with a glass rod guide. If there is space above the liquid level, fill with packing buffer. Connect the regulator to the chromatography system, start the pump to remove air bubbles from the tubing and top sieve, then pause. Insert the regulator into the column at  $45^\circ$ , fix it and tighten the seal ring to avoid air bubbles. Unscrew the bottom plug, place the bottom tubing into a waste tank, set the flow rate to 60 cm/h until the medium interface is stable, then increase to 600 cm/h and gradually reduce to 300 cm/h. Maintain this flow rate for 45 min, mark the interface with a marker, and pause the system. Screw on the bottom plug, disconnect the column top from the pump, slightly loosen the seal ring on the regulator, press the end cap 3 mm below the gel surface, and tighten the seal ring. Connect the top and bottom tubing of the column to the chromatography system for column efficiency testing.

### 2. Column Efficiency Determination and Evaluation

Column efficiency should be tested immediately after packing. Column performance is usually evaluated by theoretical plates per meter (N/m) and peak asymmetry factor (As). Higher column efficiency indicates stronger separation ability. For accurate results, the sample volume is 1.0% of

column volume, and linear flow rate is controlled at 15–30 cm/h. Load the sample close to the column inlet to minimize dilution.

| Item          | Acetone Method             | NaCl Method                 |
|---------------|----------------------------|-----------------------------|
| Sample        | 1.0% (v/v) aqueous acetone | 0.8 M NaCl aqueous solution |
| Sample volume | 1.0% column volume         | 1.0% column volume          |
| Mobile phase  | Pure water                 | 0.4 M NaCl aqueous solution |
| Flow rate     | 30 cm/h                    | 30 cm/h                     |
| Detector      | UV-280 nm                  | Conductivity                |

## 2. Column Efficiency Calculation

Calculate N/m and As from the UV (or conductivity) curve using the following formulas:  $N/m = N/L$

$$N/m = N/L$$

$$N = 5.54(V_R/W_h)^2$$

$$A_s = b/a$$

Notes: L = column height;  $V_R$  = retention volume;  $W_h$  = peak width at half-height; a = first half- peak width at 10% peak height; b = second half-peak width at 10% peak height.  $A_s$  should be between 0.8 and 1.8.  $N/m > 3000$ .

## 3. Separation and Purification

Chelating Agrose (IDA) 6FF must be chelated with metal ions before purification. Detailed chelation steps refer to 4. Regeneration.

Metal Ion Selection:  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$  are commonly used.  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  are most frequently used for purifying non-tagged proteins:  $\text{Cu}^{2+}$  binds strongly to some proteins, and certain proteins only bind  $\text{Cu}^{2+}$ ;  $\text{Zn}^{2+}$  binds weakly to some proteins and only a few proteins, often for specific protein purification.  $\text{Ni}^{2+}$  is widely used for His-tagged protein purification.  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Ca}^{2+}$  are alternatives.

Phosphate buffer is preferred at neutral to weakly alkaline pH (7.0–8.0). Avoid EDTA, citrate, etc. Tris-HCl is acceptable but not recommended when metal-protein affinity is weak. Common additives and concentrations are listed below. Low- concentration imidazole (20–40 mM) is added to binding buffer and samples to reduce non-specific adsorption of host proteins. 0.15–0.5 M NaCl must be added to eliminate ionic adsorption.

## Additives That Do Not Affect Protein Binding

| Additive                            | Common Concentration | Additive                  | Common Concentration |
|-------------------------------------|----------------------|---------------------------|----------------------|
| Phosphate, Tris, Borate, HEPES      | 20–100 mM            | Non-ionic detergents      | ≤2%                  |
| NaCl                                | ≤2 M                 | Triton X-100              | ≤2%                  |
| KCl                                 | ≤1 M                 | Tween-20                  | ≤2%                  |
| Guanidine hydrochloride             | ≤6 M                 | Octyl glucoside           | ≤2%                  |
| Urea                                | ≤8 M                 | Dodecyl maltoside         | ≤2%                  |
| Glycerol                            | ≤50%                 | C12E8, C10E6              | ≤2%                  |
| Isopropanol                         | ≤60%                 | PMSF (protease inhibitor) | 1 mM                 |
| Ethanol                             | ≤30%                 | Pepstatin                 | 1 μM                 |
| 1% Benzamidine (protease inhibitor) | ≤1 mM                | Leupeptin                 | 0.5 μg/mL            |

## Additives That May Disrupt Protein Binding (Avoid if Possible)

| Additive                                             | Common Concentration | Additive           | Common Concentration         |
|------------------------------------------------------|----------------------|--------------------|------------------------------|
| β-Mercaptoethanol                                    | -                    | Citrate            | Tolerates low concentrations |
| Reduced glutathione                                  | -                    | Histidine          | Alternative to imidazole     |
| Strong reducing agents (DTT, TCEP, DTE) <sup>①</sup> | -                    | Glycine            | —                            |
| Metal chelators (EDTA, EGTA) <sup>②</sup>            | -                    | Glutamine          | —                            |
| Ionic detergents (cholate, SDS)                      | —                    | Arginine           | —                            |
| Sodium azide                                         | 3 mM                 | NH <sub>4</sub> Cl | —                            |

## Notes:

- ① Strong reducing agents are avoided to prevent metal ion reduction and impaired protein binding. Maximum tolerance varies by sample; reducing agents only in samples, not in buffers.
- ② Metal chelators are avoided to prevent metal ion detachment from the medium. Maximum tolerance varies by sample; chelators only in samples, not in buffers.

### 3.1 Column Equilibration

Connect the column to the purification system, wash with  $\geq 5$  column volumes (CV) of binding buffer until the effluent pH and conductivity match the binding buffer. Zero the UV detector after equilibration. To reduce metal ion leaching, pre-wash with 1 CV of 0.5 M imidazole containing 1 M NaCl, then 5 CV of pure water, and equilibrate with binding buffer before loading.

Recommended binding buffer: 20 mM PB, 500 mM NaCl, 20 mM imidazole, pH 7.4, filtered through 0.2  $\mu\text{m}$  membrane. For inclusion body purification, add 8 M urea or 6 M guanidine hydrochloride to binding buffer and samples.

### 3.2 Sample Loading

Load the sample solution filtered through 0.22/0.45  $\mu\text{m}$  membrane onto the column. Loading volume depends on medium capacity, target molecule concentration, and other chromatographic conditions.

### 3.3 Column Washing

Wash with 5–10 CV of binding buffer to remove unbound impurities (until UV returns to baseline). Alternatively, follow impurity washing conditions determined in preliminary experiments.

### 3.4 Elution

Start collecting eluted fractions when the target UV peak rises. Recommended elution buffer: 20 mM PB, 500 mM NaCl, 500 mM imidazole, pH 7.4, filtered through 0.2  $\mu\text{m}$  membrane.

Elution methods: competitive elution, pH reduction, or chelator elution.

Competitive elution: Linearly or stepwise increase concentrations of metal-binding agents (0–2 M  $\text{NH}_4\text{Cl}$ , 0–0.5 M imidazole, 0–0.5 M histidine). Gradient elution at constant pH of binding buffer is recommended.

pH reduction elution: Weakly and strongly bound proteins elute sequentially as pH drops. Metal ions (e.g.,  $\text{Ni}^{2+}$ ) dissociate below pH 4.0. (If target protein is pH-sensitive, add 1/10 volume of 1 M Tris-HCl, pH 9.0 to eluate for neutralization.)

Chelator elution: 50 mM EGTA or EDTA dissociates metal ions from the medium, eluting denatured/precipitated proteins. Not generally recommended.

### 3.5 Cleaning and Storage

Wash with 3–5 CV of 0.5 M imidazole containing 1 M NaCl, rinse with pure water until effluent conductivity is 0, then wash with 2–3 CV of 20% ethanol. Store the column at 4°C. Reuse depends on sample properties; use dedicated medium per sample to avoid cross-contamination.

### 4. Regeneration

Impurity accumulation and metal ion leaching reduce performance. Regenerate and re-chelate metal ions after 1–5 cycles.

#### Steps:

Wash the column with 10 CV of pure water.

Wash with 3–5 CV of deionization buffer (20 mM PB, 0.5 M NaCl, 50 mM EDTA, pH 7.4).

Wash with 3–5 CV of binding buffer (without imidazole).

Wash with 5 CV of pure water.

Chelate with  $\geq 0.5$  CV of 0.1 M metal ion solution (e.g.,  $\text{NiSO}_4$ ) at 15 cm/h.

Wash with 3 CV of 0.5 M sodium acetate-acetic acid (pH 4.0).

Equilibrate with 5 CV of binding buffer and use immediately.

Wash with 5 CV of 20% ethanol for storage.

Metal ion solution: 0.1 M, neutral to weakly acidic pH, filtered through 0.22  $\mu\text{m}$  membrane.

Avoid phosphate buffer when chelating  $\text{Ca}^{2+}$  (precipitation risk).

For  $\text{Fe}^{3+}$  removal: incubate overnight with 50 mM EDTA ( $\text{Fe}^{3+}$  precipitates at neutral pH).

For  $\text{Fe}^{3+}$  chelation: chelate at low pH ( $\sim\text{pH } 3.0$ ) to prevent precipitation.

### 5. Cleaning-In-Place (CIP)

CIP removes strongly bound, precipitated or denatured substances. Accumulated impurities impair performance, increase backpressure and reduce flow rate. Clean after 3–5 cycles to restore capacity and flow.

Ion-exchange adsorbed proteins: Wash with 2–3 CV of 2 M NaCl, then re-equilibrate with binding buffer.

Protein precipitates/hydrophobic substances: Remove metal ions first, then soak with 0.2–1 M NaOH for  $\geq 1$  h.

Strongly bound hydrophobic proteins/lipids: Wash with 3–5 CV of 70% ethanol or 30% isopropanol (15–20 min), then re-equilibrate. Soak overnight with 6 M guanidine hydrochloride to remove most impurities. Re-equilibrate with 3–10 CV of binding buffer if used immediately.

## 6. Disinfection and Sterilization

To minimize microbial contamination:

Treat with 70% ethanol for 12 h (20% ethanol has no bactericidal/pyrogen-reducing effect).

Or soak with 0.5–1 M NaOH for 0.5–1 h.

## 7. Storage

Unused medium should be stored in a dry, well-ventilated and clean environment at 2–8°C. Ensure the container is tightly sealed; do not freeze under any circumstances. Packed chromatography columns shall be immersed and hermetically stored in 20% ethanol or 2% benzyl alcohol to prevent microbial contamination. To avoid microbial growth caused by ethanol volatilization, it is recommended to replace the storage solution with fresh one every 3 months. Prior to use, the stored medium shall undergo CIP treatment and be equilibrated with binding buffer for at least 5 column volumes (CV).

## 8. Linear Scale-Up

The purification process optimized at laboratory scale can be linearly scaled up to pilot or production scale. The following points shall be noted during scale-up:

Maintain a constant residence time to ensure stable dynamic binding capacity.

Select the column volume according to the required binding capacity; if the column height is changed, evaluate its impact on each purification step.

Determine the column diameter based on the flow rate requirement, and set the bed height according to the defined residence time. A bed height of 10–25 cm is generally recommended.

Ensure consistent sample concentration and identical elution conditions throughout the process.

## Specifications

| Attribute               | Value                                                                                                                      |
|-------------------------|----------------------------------------------------------------------------------------------------------------------------|
| Specifications & Purity | BioReagent, 70% v/v; 90µm                                                                                                  |
| Stability And Storage   | Store at 2-8°C long term (60 months). Do not freeze.                                                                       |
| Storage Conditions      | Store at 2-8°C, Do not freeze                                                                                              |
| Shipped In              | Wet ice, Do not freeze.<br>This product requires cold chain shipping. Ground and other economy services are not available. |

## Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office that best matches your location or support needs.

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- Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for hazard classification, PPE, handling, storage, and disposal.
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