CNBr-activated Agrose 4FF

Cat. No.: C1523222 | Pack size: 5ml; 25ml | Storage: Below -15℃ / -20°C

Product Information

Item	Details
Available Pack Size(s)	5ml; 25ml
Specifications & Purity	BioReagent, 70% v/v; 90μm
Stability and Storage	Store below -15℃ long term (12 months).
Storage Conditions	Store at -20°C
Shipped In	Ice chest + Ice pads. This product requires cold chain shipping. Ground and other economy services are not available.

Product Description

CNBr-activated Agrose 4FF is a pre-activated agarose medium with 4% highly cross-linked agarose as the matrix, featuring excellent physical and chemical stability. The medium enables simple, direct, rapid and mild coupling, and can directly conjugate proteins and other biological macromolecules containing primary amino groups without a coupling spacer arm. It also allows multi-point coupling, making ligands not easy to detach. Meanwhile, it has strong hydrophilicity, low non-specific adsorption, wide universality and application scope, fast flow rate, good repeatability and easy process scale-up. It is mainly used for the separation and purification of biological molecules such as proteins.

Aladdin CNBr-activated Agrose 4FF is stored in acetone, with a volume ratio of gel to protective solution of 7:3. The product specification is based on the actual gel volume.

Parameter Specifications

Item	Specification
Matrix	4% Highly cross-linked agarose
Coupling functional group	$-NH_2$
Activated group	CNBr

Item	Specification
Average particle size	90 μm
Coupling conditions	pH 8-10, room temperature (20-25℃) for 2-3 h or 4℃ overnight
Coupling capacity	13-26 mg α-chymotrypsinogen/mL medium
Recommended flow rate	<250 cm/h
Recommended pressure	<0.15 MPa (1.5 bar)
pH stability ①	3-11 (long-term); 2-11 (short-term)
Chemical stability	Stable in common aqueous buffers after ligand coupling; denaturants such as 8 M urea or 6 M guanidine hydrochloride can be used ②
Storage conditions	Acetone; below -15℃
Shelf life	1 year

Notes: ① Long-term refers to the pH range where the medium remains stable for a long time without adverse effects on its subsequent performance. Short-term refers to the pH range for medium regeneration, cleaning-in-place and disinfection based on experience.

② The stability after ligand coupling is premised on the ligand's tolerance to changes in pH or chemical environment.

Instructions for Use

1. Coupling of Functional Ligands

1.1 Buffer Volume

Solution	Composition	Volume Ratio
Solution A	1 mM HCl, 0.5 M NaCl (pre-cooled, pH 2-3)	≥20× gel volume
Solution B	0.2 M $NaHCO_3$, 0.5 M NaCl, pH 8.3	≥10× gel volume
Solution C	0.1 M Tris-HCl, pH 8.0	≥5× gel volume
Solution D	0.05 M Tris-HCl, 0.5 M NaCl, pH 8-9	≥15× gel volume
Solution E	0.1 M Acetic acid-sodium acetate, 0.5 M NaCl, pH 3-4	≥15× gel volume
Solution F	0.02 M PB, 0.15 M NaCl, pH 7.0-7.6	≥10× gel volume

1.2 Dissolve dry powder protein ligand with Solution B; replace the buffer of solution-state protein ligand with Solution B.

1.3 Place an appropriate amount of affinity chromatography medium stored in acetone in a glass filter funnel, and wash with 15× gel volume of pre-cooled Solution A.

1.4 After washing, add the medium to the ligand solution, mix well, and react at room temperature for 2-4 h or at 4℃ overnight.

1.5 After coupling, wash with 5× gel volume of Solution B to remove excess uncoupled ligand.

1.6 Wash with 3× gel volume of Solution C, then continue to block remaining active sites of the medium with Solution C at room temperature for 2-4 h.

1.7 Wash alternately with Solution D and Solution E for 3-6 cycles, 3× gel volume each time.

1.8 Finally, wash with 10× gel volume of Solution F to complete ligand coupling.

Coupling Notes

Coupling buffer: Bicarbonate or borate buffer is commonly used. Do not use Tris or other amino-containing buffers as coupling buffer; pH 8-10 is recommended for high coupling efficiency. Low pH affects coupling activity, but pH 6 may be used to reduce steric hindrance or preserve ligand bioactivity.

Add 0.5 M NaCl to reduce protein-protein adsorption.

Temperature & time: 2-3 h at room temperature (25℃); overnight (10-16 h) at 4℃.

Ligand concentration: 5-10 mg protein per mL medium is recommended. Excess ligand causes high ligand density, steric hindrance, reduced binding capacity, difficult elution and increased non-specific adsorption.

2. Column Packing

2.1 Packing Buffer Preparation: Purified water, ultrasonic degassing for 15 min.

2.2 Chromatography Medium Preparation: Calculate required medium volume (compression factor ~1.15) and weigh. Replace buffer to packing buffer via vacuum filtration. Prepare ~75% gel suspension with packing buffer.

2.3 Chromatography Column Preparation: Inspect column for integrity and cleanliness. Install lower end cap, tighten O-ring, fix column vertically and verify with level gauge. Remove air bubbles from bottom sieve with packing buffer via syringe, then cap. Add ~2 cm height of packing buffer to column.

2.4 Column Packing (Example: 16 mm diameter, 15 cm bed height): Mix gel suspension, pour slowly into column with glass rod; fill with packing buffer if needed. Connect regulator to system, purge air bubbles at set flow rate then pause. Insert regulator at 45° and seal. Open bottom cap, set flow rate to 60 cm/h until bed interface stabilizes, then increase to 400 cm/h for 45 min, mark interface. Pause

system, cap bottom, disconnect top, loosen regulator seal, lower upper end cap to 2 mm below gel surface, retighten. Connect column to system for column efficiency test.

3. 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

Column Efficiency Calculation: Calculate N/m and As from the UV (or conductivity) curve using the following formulas:

$N/m = N/L$

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

$As = b/a$

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. As should be between 0.8 and 1.8. N/m > 3000.

4. Separation and Purification

4.1 Column Equilibration: Flush column with binding buffer for ≥3-5 CV until effluent pH and conductivity match binding buffer; zero UV detector.

4.2 Sample Loading: Load sample filtered through 0.2/0.45 µm membrane. Loading volume depends on medium capacity, target molecule concentration and conditions.

4.3 Column Washing: Wash with binding buffer for 3-5 CV until UV 280 returns to baseline (or use optimized washing conditions).

4.4 Elution: Elute with elution buffer; collect fractions when target UV peak appears.

4.5 Cleaning and Storage: Regeneration: Alternate high-pH (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) and low-pH (0.1 M sodium acetate-acetic acid, 0.5 M NaCl, pH 4.5) buffers for 3-5 cycles (2-3 CV each).

Equilibrate with binding buffer (3-5 CV), flush with pure water to zero conductivity, then flush with 20% ethanol (2-3 CV). Store at 2-8℃.

Conditions vary by ligand; all buffers must be 0.22 μm filtered. Sample buffer should match equilibration buffer.

5. Cleaning-In-Place (CIP)

CIP removes strongly bound, precipitated or denatured contaminants that impair performance, increase backpressure and reduce flow rate. Recommended every 1-5 cycles.

Precipitated/denatured contaminants: 0.1 M NaOH (4 CV, 1-2 h contact) → binding buffer (≥5 CV); or 6 M guanidine hydrochloride (2 CV) → immediate binding buffer wash (≥5 CV).

Hydrophobic impurities: 0.1-0.5% non-ionic detergent (2 CV) → binding buffer (≥5 CV); or 70% ethanol (3-4 CV) → immediate binding buffer wash (≥5 CV).

6. Sterilization

Minimize microbial contamination with 70% ethanol (if ligand-tolerant) for 12 h, then flush with sterile binding buffer (≥5 CV).

7. Storage

Unused medium: Store below -15℃, protected from light, dry, ventilated and sealed; short-term storage at 4℃ (light-proof).

Coupled medium/column: Store sealed in 20% ethanol or 2% benzyl alcohol. Replace storage solution every 3 months to prevent microbial growth.

8. Linear Scale-Up

Lab-scale purification can be linearly scaled to pilot or production scale with these guidelines:

- Keep residence time constant for stable dynamic binding capacity.
- Select column volume based on binding demand; adjust bed height if needed.
- Determine column diameter by flow rate; recommended bed height: 3-15 cm.
- Maintain uniform sample concentration and consistent elution conditions.

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