

## Con A Agarose Resin

### C1492535

#### Storage:

- Store in 0.1 M acetate, 1 M NaCl, 1 mM CaCl<sub>2</sub>, 1 mM MnCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, pH 6.0, with 20% ethanol as preservative.
- Store at 2–8°C. Do not freeze.

#### Introduction:

This product is a bioaffinity chromatography separation medium formed by covalently coupling Concanavalin A (Con A) to agarose gel microspheres. It is primarily used for the separation and purification of various glycoproteins (including glycoprotein viruses), polysaccharides, and glycolipids containing mannose and glucose residues. Con A, extracted from jack beans, is a tetrameric metalloprotein and a type of lectin. In the presence of Mn<sup>2+</sup> and Ca<sup>2+</sup>, it binds to α-D-mannopyranosyl, α-D-glucopyranosyl, and molecules with spatially related C-3, C-4, or C-6 hydroxyl residues. Applications of Con A affinity chromatography medium also include purification of enzyme-antibody conjugates, IgM purification, isolation of detergent-solubilized cell surface glycoproteins from membranes, separation of membrane vesicles, and studies of changes in carbohydrate-containing substances.

Aladdin Con A Agarose Resin is stored in 20% (v/v) Ethanol, 0.1 M acetate, 1 M NaCl, 1 mM CaCl<sub>2</sub>, 1 mM MnCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, pH 6.0, with a settled gel to storage solution ratio of 1:1. The product specification refers to the actual volume of the settled gel.

Parameter	Value / Description
Matrix	Cross-linked Agarose
Ligand	Con A, ≥8 mg/mL
Particle Size Range <sup>①</sup>	45~165 μm
Average Particle Size	~90 μm
Binding Capacity <sup>②</sup>	≥30 mg Thyroglobulin/mL
Recommended Operating Flow Rate <sup>③</sup>	15~150 cm/h
Maximum Flow Rate & Pressure <sup>④</sup>	900 cm/h, 0.3 MPa
Operating pH Range	pH 4~9
Chemical Stability	Stable in common aqueous buffers. Avoid chelators (e.g., EDTA), 8 M urea, or solutions below pH 3, as they may remove essential metal ions and inactivate the lectin.
Storage Conditions	20% (v/v) Ethanol, 0.1 M acetate, 1 M NaCl, 1 mM CaCl <sub>2</sub> , 1 mM MnCl <sub>2</sub> , 1 mM MgCl <sub>2</sub> , pH 6.0; 2–8°C
Shelf Life	3 years

## Notes:

- ① Over 90% of the beads fall within this size range.
- ② Binding capacity measured by incubating with 2 mg/mL porcine thyroglobulin for 2 hours; this value is close to the dynamic binding capacity ( $DBC_{10\%}$ ).
- ③ This flow rate range is recommended for optimal binding performance but is not an absolute operating limit.
- ④ Maximum tested flow rate at 10 cm column height.

## Protocol:

### 1. Column Packing

The following procedure describes column packing when connected to a chromatography system:

- 1) Equilibrate all materials to the operating temperature. Degas liquids if possible.
- 2) Resin Quantity Calculation:

Settled resin volume = Column volume × Compression factor (1.15 for this resin).

- 3) Resin Preparation: Resuspend the resin slurry thoroughly. Measure the required volume, remove storage solution by filtration, and wash 3 times with ~3 resin volumes of purified water.
- 4) Packing Slurry Preparation: Transfer resin to a suitable container and add packing solution to achieve a 50–75% (v/v) slurry. Mix well before use.
- 5) Pre-packing Setup: Fill the bottom of the clean column with packing solution to remove air from the bottom frit and outlet. Retain a small amount of liquid, tighten the bottom end fitting, and ensure the column is vertical.
- 6) Packing: Pour the well-mixed slurry into the column in one slow, continuous motion (use a packing reservoir if needed). After adding all resin, fill the reservoir with packing solution, attach the top lid, and connect the column to the system.
- 7) Compression: Allow the resin to settle naturally (or use a low flow rate of 10–20 cm/h). Apply a high flow rate (300–600 cm/h; ensure pressure  $\leq 0.3$  MPa) until the interface is stable. Mark the stable height, stop the pump, and lower the adapter to the position corresponding to the compression factor (1.15). Tighten the adapter and equilibrate the column at a high flow rate.

Packing Condition	Con A Agarose Resin
Compression Factor	1.15
Packing Flow Rate	300-600 cm/h

### 2. Column Efficiency Testing

After packing, assess column efficiency using HETP (Height Equivalent to a Theoretical Plate) and As (Asymmetry Factor) with acetone or NaCl as tracers:

Tracer	1.0% Acetone	0.8~1.0 M NaCl
Sample Volume	1.0% CV	1.0% CV
Mobile Phase	Pure Water	0.4 M NaCl
Flow Rate	30 cm/h	30 cm/h
Detector	UV-280 nm	Conductivity

Calculate HETP, N, and As using:

- $HETP = L / N$
- $N = 5.54 \times (V_R / W_n)^2$
- $As = a / b$

HETP should be  $<3 \times$  the average particle diameter ( $HETP/D_{50} < 3$ ), and As should be 0.8–1.5.

### 3. Equilibration and Loading:

- Recommended Binding Buffer (Buffer A): 20 mM Tris-HCl, 500 mM NaCl, 1 mM  $MnCl_2$ , 1 mM  $CaCl_2$ , pH 7.4. If higher concentrations of  $Mn^{2+}$  and  $Ca^{2+}$  (e.g., 5 mM) are used during equilibration, they may be omitted from the binding buffer. The pH of the binding buffer should be between 6.5–7.5.
- Equilibrate the column with 5–10 CV of Buffer A at the operating flow rate until baseline (conductivity/pH) stabilizes.
- Load sample at 50–80% of  $DBC_{10\%}$ . For initial testing, reduce the load to observe binding, specificity, and elution. Pre-treat sample by centrifugation ( $\geq 10,000$  g) and filtration (0.22/0.45  $\mu m$ ).
- Wash with 3–10 CV of Buffer A after loading.

### 4. Elution:

- Common Elution Buffer: 0.1–0.5 M methyl- $\alpha$ -D-glucopyranoside (methyl- $\alpha$ -D-glucoside) or methyl- $\alpha$ -D-mannopyranoside (methyl- $\alpha$ -D-mannoside) in 20 mM Tris-HCl, 500 mM NaCl, pH 7.4.
- Most glycoproteins elute at 0.1–0.2 M sugar concentration. Tightly bound substances may require higher concentrations. For initial use, try a gradient elution from 5–500 mM.
- Alternatively, lower the pH (but not below 4.0) or use weaker eluents like glucose or mannose.
- Tip: Pausing the flow for a few minutes during elution can improve glycoprotein recovery.

### 5. Regeneration:

- Before reuse, clean with 5–10 CV of 20 mM Tris-HCl, 0.5 M NaCl, pH 8.5, followed by equilibration with 2–5 CV of binding buffer.
- Optionally, include an intermediate step: 2–5 CV of 20 mM NaAc, 1 M NaCl, pH 4.5.
- For strongly adsorbed substances, use 0.1 M borate buffer (pH 6.5–8.5) at a low flow rate for 3–5 CV. Borate forms complexes with cis-diol groups of sugars, enabling competitive elution.