TA-CNBr 4B

Pre-activated Agarose Gel Resins
User Manual

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1. Introduction

TA-CNBr 4B is a cyanogen bromide preactivated agarose gel. Biomolecules with primary amino groups (-NH_2) can be covalently coupled to this medium for making specific affinity media as well as immobilised enzymes.

This resin features:

· Mild reaction conditions with biomacromolecules such as proteins

· Direct coupling of biomacromolecules without coupling spacer arms

· The ligand and the base can form multi-site coupling, preventing the ligand from fall off

· Applicable to the coupling of proteins, peptides, nucleic acids, etc.

Product Specifications

Product	TA-CNBr 4B
Appearance	White slurry, forms visible layering upon standing / White powder
Base Matrix	4% agarose
Functional Group	Amino
Particle Size Range	45–165 μm
Amount of Coupled Protein	25–60 mg of α-chymotrypsinogen per 1 mL of packing material
Max. Pressure Tolerance	0.16 bar
Chemical Stability	Stable in common aqueous solutions: 8M urea, 6 M guanidine hydrochloride, 70% ethanol
pH Stability	3–11 (operating); 2–11 (CIP, short-term)
Storage Conditions	2–8 °C, 100% acetone

2. Instructions to Use

Ligand Coupling:

TA-CNBr 4B is supplied either in acetone or as a dry powder (1 g dry powder swells to approximately 3.5 mL wet resin in 1 mM HCl). Prior to ligand coupling, the protective agents (e.g., acetone) must be removed, and dry powder must be fully swollen.

Coupling Solution A: 1 mM HCl.

Coupling Solution B: 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3

Procedure:

1. Transfer the resin (preserved in acetone) into a sintered glass funnel. (If using dry powder, pre-swell in 1 mM HCl for 10–15 min.)
2. Wash thoroughly with pre-chilled Coupling Solution A (0–4 °C) for at least 30 minutes. Use approximately 60 mL of Solution A per 1 mL of resin.
3. Dissolve the ligand in Coupling Solution B or buffer-exchange it into Solution B using a TD-G25 desalting column. Recommended ligand concentration: 5–10 mg resin per mL of packing material.
4. Dilute the washed resin with Coupling Solution A (0.5 mL per 1 mL of resin), then mix with an equal volume of the ligand solution. Stir gently at room temperature for 2 hours or at 4 °C overnight. (Note: Do not use a magnetic stirrer.)
5. Blocking: After coupling, remove the supernatant and add blocking buffer (0.1 M Tris-HCl, pH 8.3). Incubate at room temperature for 2–4 hours.
6. Washing: Wash the resin alternately with the following buffers 3–6 times using 5 resin volumes per wash:
   a) 0.1 M Tris-HCl + 0.5 M NaCl, pH 8–9
   b) 0.1 M acetate buffer + 0.5 M NaCl, pH 3–6
7. After washing, equilibrate with PBS or store in 20% ethanol (provided the coupled ligand is stable in ethanol).

Column Packing

Note: The coupled resin must be packed into a chromatography column before use in purification workflows.

· Determine the required amount of TA-CNBr 4B based on the column volume:

Settled bed volume = Column volume × 1.15 (i.e., packing compression factor = 1.15)

· Buffer exchange of the storage solution:

Pour the resin slurry into a Büchner funnel and remove the liquid under vacuum. Wash the resin with approximately 3 volumes of distilled water.

(Alternative method: For larger volumes or limited equipment, allow the slurry to settle, decant the supernatant, add an equal volume of distilled water, mix thoroughly, and let it settle again. Repeat this washing process 2–3 times.)

· Slurry preparation:

Add 0.5–1.0 volumes of distilled water per volume of settled resin, mix thoroughly.

· Prepare a clean TK-EC chromatography column.

(TK-EC series columns are available in diameters from 1 cm to 45 cm, suitable for a wide range of purification scales.)

Remove any air bubbles trapped under the bottom frit and maintain approximately 1 cm of water in the column. Ensure the column is positioned vertically.

· Pour the prepared slurry into the column in one go, slowly and carefully to avoid introducing air bubbles. If necessary, use a packing reservoir. After pouring, stir gently with a resin spatula to ensure uniform distribution.

· Attach the top adapter to the chromatography system or peristaltic pump. Remove air bubbles trapped below the top frit.

(For columns with diameters less than 20 cm, you may invert the adapter and use a peristaltic pump or pipette bulb to draw out the air beneath the frit.)

Insert the adapter into the column and gently agitate it to release any bubbles around the edges. Tighten the sealing knob.

(For columns >30 cm in diameter, do not tighten the O-ring completely at first. Press the adapter down to allow liquid to flow out through the adapter, displacing air. Then tighten the sealing knob.)

· Set the packing flow rate to 1.3 times of operating flow rate. Open the column outlet and start the pump. After the resin has fully settled, continue the flow for at least 3 column volumes.

Mark the height of the packed bed, then stop the pump.

· Remove packing reservoir if used. Press the adapter down to approximately 0.3 cm below the marked bed height. Column packing is now complete.

Column Efficiency Evaluation

Column efficiency can be assessed using either acetone or NaCl as a tracer. Prepare the tracer solution and mobile phase according to the table below:

Method	Acetone Method	NaCl Method
Sample	1.0% (v/v) acetone in water	0.8 M NaCl in water
Sample Volume	1.0% of column volume	1.0% of column volume
Mobile Phase	Water	0.4 M NaCl in water
Flow Rate	30 cm/h	30 cm/h
Detection	UV at 280 nm	Conductivity

Column Efficiency Calculation

Calculate the height equivalent to a theoretical plate (HETP), number of theoretical plates (N), and asymmetry factor (As) based on the UV or conductivity chromatogram using the following formulas:

- HETP = L / N
- $N = 5.54 \times (VR / Wh)^2$

  Where:

  VR = retention volume

  Wh = peak width at half height

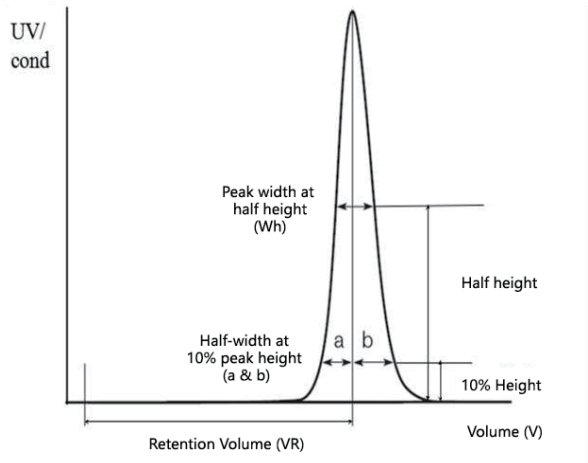
  L = column bed height

  (VR and Wh must use the same units)
- As = b / a

  Where:

  a = front half-width at 10% peak height

  b = tail half-width at 10% peak height



Result Evaluation

$h = HETP / d_{50v}$

Where d_{50v} is the median particle diameter by volume (in cm).

In general, column efficiency is considered acceptable when:

· HETP is less than 3, and

· Asymmetry factor falls within the range of 0.8–1.8

If the efficiency does not meet these criteria, the potential causes should be investigated, and the column should be repacked.

Chromatography Method

· Sample Preparation:

For complex protein mixtures, the sample concentration should not be too low, as lower concentrations may reduce binding efficiency. However, if the target molecule binds specifically to the ligand on the resin, sample concentration is less critical.

Avoid overly high sample concentrations (>30 mg/mL), as these can cause pH and ionic strength fluctuations, negatively impacting binding. In such cases, dilute the sample with the binding buffer.

High-viscosity samples may cause uneven flow rates during chromatography and should be avoided.

Prior to loading, clarify the sample by centrifugation or filtration through a 0.45 μm membrane to prevent clogging and maintain column performance and lifespan.

· Binding Buffer:

The pH, salt concentration, and temperature of the binding buffer should be optimized based on the interaction conditions between the ligand and the target molecule.

· Flow Rate:

Use a flow rate of less than 50 cm/h, depending on binding strength between the target and the ligand. Weaker interactions require slower flow rates to ensure efficient binding.

· Sample Load:

Determined by the properties of the ligand and the target molecule.

· Washing:

Determined by the properties of the ligand and the target molecule.

· Elution:

Select an elution buffer based on the nature of the ligand-target interaction. Common elution strategies include:

1. pH shift elution, for antigen–antibody interactions
2. Competitive elution, for enzyme–substrate interactions

3. Cleaning and Regeneration

Routine in-place cleaning is essential to prevent buildup and maintain consistent performance. In-place cleaning should be performed after every use to maintain reproducibility.

The regeneration solution must be compatible with the stability of the coupled ligand. Only use cleaning agents if the ligand is confirmed to be stable under those conditions.

Recommended regeneration protocol:

· Wash the resin alternately with the following buffers 3–6 times using 5 resin volumes per wash:

a) 0.1 M Tris-HCl + 0.5 M NaCl, pH 8–9

b) 0.1 M acetate buffer + 0.5 M NaCl, pH 3–6

· Denaturants: 8 M urea, 6 M guanidine hydrochloride

· Organic solvents: 70% ethanol, 30% isopropanol

4. Sterilization and Storage

Sterilization: If the ligand is stable in ethanol, the coupled TA-CNBr 4B resin can be sterilized by soaking in 70% ethanol for 12–24 hours.

Storage: Uncoupled TA-CNBr 4B should be sealed in 100% acetone at 4–8 °C for storage. Coupled TA-CNBr 4B can be stored in 20% ethanol.

To minimize ethanol evaporation and microbial growth, it is recommended to replace the storage solution every 3 months.

5. Disposal and Recycling

TA-CNBr 4B is not readily biodegradable in the environment. To minimize environmental impact, disposal by incineration is recommended.

6. Ordering Information

Product	P/N	Specification
TA-CNBr 4B	Y5148	25g
	Y5149	100g
	Y5150	500g
	Y5151	1kg
	Y5152	5kg
	Y5153	10kg
	Y5154	25mL
	Y5155	100m
	Y5156	500mL
	Y5157	1L
	Y5158	5L
	Y5159	10L
	Y5160	20L