

## Q Agarose (High Resolution)

Cat. No.: Q1523866 | Pack size: 25 mL, 100 mL | Storage: 2-8 °C; do not freeze

### Overview

Ion Exchange Chromatography (IEC) is a chromatographic method that uses ion exchange ligands as the stationary phase. Separation is achieved based on the difference in binding strength during the reversible exchange between component ions in the mobile phase and counter ions on the stationary phase. Featuring high resolution, large binding capacity and easy operation, IEC has become one of the most widely used purification techniques for the separation and purification of biochemical products, proteins, peptides and other biomolecules. Ion exchange media mainly consist of three parts: a cross- linked network framework (i.e. base matrix), functional groups immobilized on the framework, and mobile counter ions with opposite charges to the functional groups that participate in ion exchange.

Q Agarose (High Resolution) is prepared by chemical modification of highly rigid agarose beads with an average particle size of 40  $\mu\text{m}$ . It delivers improved mechanical properties and excellent separation performance at elevated flow rates.

With a small particle size and superior resolution, this product also combines high flow rate, high pressure resistance and low back pressure, making it suitable for intermediate purification and polishing of biomolecules.

Aladdin Q Agarose (High Resolution) is stored in 20% ethanol, with a volume ratio of gel to preservative solution of 7:3. The product specification of our company refers to the actual volume of the gel.

**Table 1 Product Specifications of Q Agarose (High Resolution)**

| Parameter             | Specification                                 |
|-----------------------|-----------------------------------------------|
| Matrix                | High-rigidity Agarose                         |
| Ligand                | Quaternary                                    |
| Mean particle size    | 40 $\mu\text{m}$                              |
| Ion Capacity          | 150-180 $\mu\text{mol Cl}^-/\text{mL medium}$ |
| Recommended Flow Rate | <300 cm/h                                     |
| Recommended Pressure  | <0.5 MPa (5 bar)                              |
| Working pH Range ①    | 2-12                                          |
| pH Stability          | 2-12 (Long-term) 2-14 (Short-term)②           |

| Parameter          | Specification                                                                                                                   |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------|
| Chemical Stability | Stable in common buffers, such as 1 M NaCl, 1 M NaOH, 6 M guanidine hydrochloride, 8 M urea, 70% ethanol, 30% isopropanol, etc. |
| Storage            | 20% ethanol, 2-8 °C                                                                                                             |
| Shelf Life         | 5 years                                                                                                                         |

### Notes

- ① Working pH range: the pH range within which the separation and purification function can be achieved under the premise of stable chromatography medium.
- ② Long-term pH range: the pH range within which the medium remains stable for a long time without adverse effects on its subsequent performance. Short-term pH range: the empirically determined pH range for medium regeneration, cleaning in place and sanitization.

## Instructions for Use

### 1. Column Packing

#### 1.1 Preparation of Packing Buffer

Purified water, subjected to ultrasonic degassing for 15 minutes.

#### 1.2 Preparation of Chromatography Medium

Calculate the required amount of chromatography medium (compression factor is approximately 1.15), weigh and set aside. Perform buffer exchange into the packing buffer using a vacuum filter flask. Add a certain amount of packing buffer to the exchanged medium to prepare a slurry with a concentration of approximately 50%.

#### 1.3 Preparation of Chromatography Column

Inspect the chromatography column to ensure all components are intact and clean. Install the bottom column end fitting, tighten the O-ring, then vertically fix the chromatography column on an iron stand. Check and adjust the column with a spirit level to ensure it is perfectly vertical. Draw the packing buffer into a syringe, connect it to the outlet at the bottom of the column, slowly push out the liquid in the syringe to remove air bubbles from the bottom frit. Remove the syringe and screw on the end cap. Add packing buffer to the column to a height of approximately 2 cm.

#### 1.4 Column Packing (taking a chromatography column with 16 mm inner diameter and 10 cm bed height as an example)

Mix the slurry thoroughly, and slowly pour it into the chromatography column guided by a glass rod. If there is a gap between the liquid level and the top of the column, fill it with packing buffer. Connect the flow adapter to the chromatography system, start the pump at a certain flow rate to remove air bubbles from the tubing and the top frit, then pause. Place the flow adapter into the column at a 45° angle, fix it in place and tighten the seal ring, taking care to avoid air bubbles entering. Unscrew the end cap at the bottom of the chromatography column, place the bottom tubing into the waste container, set the flow rate to 60 cm/h. After the medium interface remains unchanged, set the flow rate to 1200 cm/h and maintain this flow rate for 45 minutes. Mark the interface with a marker pen and pause the system. Screw on the bottom end cap, disconnect the upper end of the column from the pump, slightly loosen the seal ring on the flow adapter, press the column end fitting to 1-3 mm below the gel surface, and tighten the seal ring. Connect the top and bottom tubing of the chromatography column to the chromatography system for column efficiency test.

#### 2. Column Efficiency Test

After column packing is completed and before use, the packing quality of the chromatography column can be confirmed through column efficiency measurement and evaluation. Column efficiency is usually evaluated by the Height Equivalent to a Theoretical Plate (HETP) and the Asymmetry factor (As). Acetone or NaCl can be used as the sample for column efficiency measurement, and the sample solution and mobile phase are prepared according to the following table.

**Table 2 Solution Preparation for Column Efficiency Test**

| Test Sample | Sample Solution Preparation          | Mobile Phase Preparation | Detection Method       |
|-------------|--------------------------------------|--------------------------|------------------------|
| Acetone     | 0.1% (v/v) acetone in purified water | Purified water           | UV detection at 280 nm |
| NaCl        | 1 M NaCl in purified water           | Purified water           | Conductivity detection |

Calculate the Height Equivalent to a Theoretical Plate (HETP), number of theoretical plates (N) and Asymmetry factor (As) according to the UV or conductivity curve, using the following formulas:

$$\text{HETP} = L / N$$

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

As = a / b Where: L is the column height;

$V_r$  is the retention volume;  $W_h$  is the peak width at half height; a is the first half peak width at 10% of the peak height; b is the second half peak width at 10% of the peak height. In general, the HETP value should be less than three times the average particle size of the packing material (i.e.,  $\text{HETP}/D_{50} < 3$ , where  $D_{50}$  is the average particle size of the packing material), and As should be between 0.8 and 1.5.

### 3. Separation and Purification

#### 3.1 Column Equilibration

Connect the chromatography column to the purification equipment, and rinse with binding buffer for more than 3-5 column volumes (CV) until the pH and conductivity of the column effluent are consistent with those of the binding buffer. Zero the UV detector after equilibration is completed.

#### 3.2 Sample Loading

Load the sample solution filtered through 0.22/0.45  $\mu\text{m}$  membrane onto the ion exchange chromatography column. The loading volume depends on the binding capacity of the chromatography medium, the concentration of the molecules bound to the medium in the sample solution, and other chromatographic conditions.

#### 3.3 Column Washing

Rinse with binding buffer for 3-5 CV to remove impurities that do not bind to the medium under this condition (until the UV 280 detection value returns to near the baseline). Alternatively, perform column washing according to the impurity elution conditions determined in previous experiments.

#### 3.4 Elution

Rinse the chromatography column with elution buffer, and start collecting the eluted sample when the target UV peak rises.

#### 3.5 Washing and Storage

Wash with 1-2 M NaCl for 5 CV to remove reversibly bound impurities, then rinse with purified water until the conductivity of the effluent is 0, and finally rinse with 20% ethanol for 2-3 CV. Store the chromatography column in an environment at 2- 8°C. Do not freeze.

### 4. Cleaning In Place (CIP)

Cleaning In Place (CIP) is the process of removing strongly bound, precipitated or denatured substances from the chromatography medium. Residual impurities will affect the chromatographic performance of the column. Severe aggregation will clog the chromatography column, increase back pressure and reduce flow rate. Therefore, regular CIP can prevent the accumulation of contaminants in the column bed and help maintain the binding capacity and flow rate of the medium. CIP should be performed after the chromatography column has been used for 3-5 cycles to restore the medium performance. Inorganic salts, acids, bases or organic solvents are usually used for cleaning. The recommended cleaning conditions for different types of impurities and contaminants are as follows:

#### 4.1 Removal of impurities adsorbed by ion exchange

Wash the chromatography column with 2-3 CV of 2 M NaCl solution, then rinse the column with 3-5 CV of purified water.

#### 4.2 Removal of protein precipitates and hydrophobic impurities

Soak the chromatography column in 1 M NaOH for more than 1 hour, then rinse the column with 5-10 CV of purified water.

#### 4.3 Removal of strongly bound hydrophobic impurities

Rinse the chromatography column with 3-5 CV of 70% ethanol or 30% isopropanol for 15-20 minutes, then rinse the column with 3-5 CV of purified water.

#### 4.4 Removal of nucleic acids

After rinsing with 1-2 CV of neutral buffer, wash with 2-5 CV of 0.1 M acetic acid at pH 3.0, then perform reverse flow washing with 1 M NaOH for 15-30 minutes. If the column is to be used immediately after cleaning, simply rinse with binding buffer for 3-10 CV.

#### 5. Sanitization and Sterilization

Sanitization and sterilization are performed to minimize microbial contamination in the chromatography column. NaOH solution can be used as a disinfectant for this product. NaOH solution can effectively remove viruses, bacteria, yeast and endotoxins with extremely low operating costs. The sanitization steps are as follows:

5.1 Rinse the chromatography column with binding buffer for 3-5 CV.

5.2 Rinse the chromatography column with 0.5 M NaOH solution for 2-5 CV.

5.3 Soak the chromatography column in 0.5-1 M NaOH solution for 1 hour.

5.4 Rinse the chromatography column with binding buffer with pH between 7 and 8 for 5-10 CV to complete the sanitization process.

**Precautions:** When the chromatography column is severely contaminated, use 0.5 M NaOH mixed with 30-40% propanol for cleaning. High concentration of NaOH or prolonged NaOH treatment will reduce the binding capacity of the medium. Please pay attention to the cleaning concentration and time during sanitization.

#### 6. Storage

Unused medium should be stored in a dry, ventilated and clean environment at 2-8 °C, ensure the container mouth is completely sealed, and never freeze. Pre-packed chromatography columns should be soaked and sealed in 20% ethanol or 2% benzyl alcohol for storage to prevent microbial contamination.

#### 7. Linear Scale-up

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

Keep the residence time unchanged to ensure the stability of the dynamic binding capacity.

Select the column volume according to the required binding capacity. If the column height is changed, pay attention to whether it will affect the purification steps.

Determine the column diameter according to the flow rate requirement, and determine the bed height according to the known residence time. The recommended bed height is generally 10-25 cm.

Ensure the sample concentration is uniform and consistent, and the elution conditions are the same.

## Specifications

|                                    |                                                                                                                         |
|------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| <b>Specifications &amp; Purity</b> | BioReagent, 70% v/v; 40 µm                                                                                              |
| <b>Stability and Storage</b>       | Store at 2-8 °C long term (60 months). Do not freeze.                                                                   |
| <b>Storage</b>                     | Store at 2-8 °C; do not freeze                                                                                          |
| <b>Shipped In</b>                  | Wet ice; do not freeze. 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 for your region; for general inquiries, the North American office is the corporate primary.

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## Limitations & Disclaimer

- ☐ For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
- ☐ This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
- ☐ 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 handling, storage, and disposal information.
- ☐ Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the kit for their specific application.
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