

# Technical note

## J.T. Baker® BAKERBOND® PolyQUAT™ multimode strong anion exchange chromatography resin

### INTRODUCTION

Utilizing a unique surface chemistry combining strong anion exchange functionality with weak anionic exchange sites, BAKERBOND® PolyQUAT™ multimode anion exchange chromatography media provides a unique selectivity to enable higher purification performance across a wide range of biopharmaceuticals.

Designed and manufactured by Avantor to the high standards established by our J.T. Baker® brand, the BAKERBOND® PolyQUAT™ resin provides better selectivity than conventional strong anion exchange media that enables separation of proteins and peptides having similar isoelectric points (pI). Its fully porous and crosslinked methacrylate spherical beads provide excellent mechanical and chemical stability and are optimized for maximum capacity, high mass transfer, and relatively low backpressure. The resin is provided in a non-hazardous, non-flammable storage solution through Avantor's global supply chain.

Used with Avantor's proven J.T. Baker® family of process chromatography buffers and additives, the BAKERBOND® PolyQUAT™ resin can deliver greater efficiencies and higher purity profiles to biopharma chromatography schemes.

### FEATURES

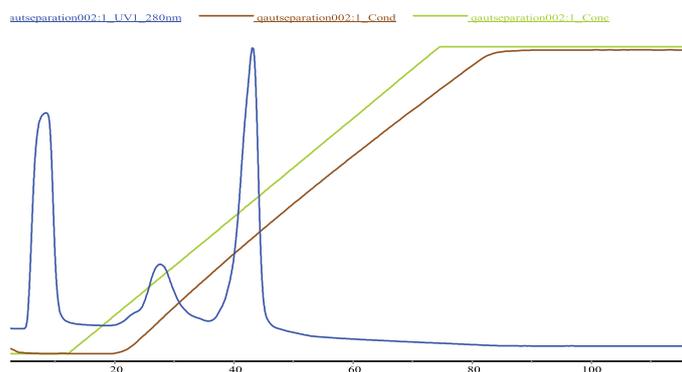
- Unique selectivity delivered by its proprietary mixed mode functionality
- High ionic capacity to improve separation and enable operation at high salt concentration
- Spherical polymethacrylate beads enable uniform packing and provide mechanical stability and chemical resistance for ease-of-use
- Delivery in a non-hazardous, non-flammable buffer solution to eliminate burdensome handling requirements.



## UNIQUE SELECTIVITY

BAKERBOND® PolyQUAT™ anion exchange resin is inherently well suited to discriminate between extremely similar molecules. Its unique surface chemistry is able to discern minute differences in primary structure resulting in a unique selectivity, improved separation performance, and higher efficiency downstream operations. As illustrated in Fig.1, molecules with similar pI such as Cytochrome C, rabbit IgG, and β-lactoglobulin are well resolved and can be easily separated.

Fig.1. Isolation of IgG from Cytochrome C and β-lactoglobulin mixtures



Column: 10 × 100 mm

Buffer A: 50 mM TRIS, pH 8.5

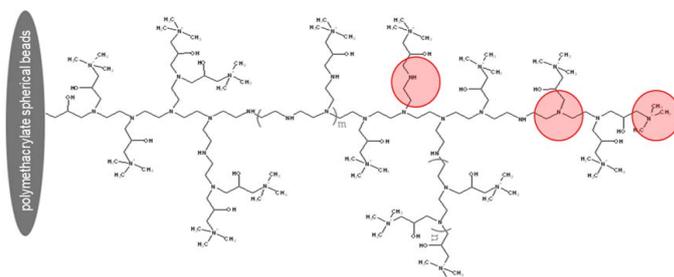
Buffer B: Buffer A plus 1.0 M NaCl, pH 8.5 Linear gradient from 0 to 100% B in 10 CV

Flow rate: 5 mL/minute

Sample volume: 3.9 mL.

Protein	pI	Sample conditions
Cytochrome C	10.5	0.3 mg/mL
Rabbit IgG	7.2	0.2 mg/mL
β-Lactoglobulin	5.4	0.7 mg/mL

The ability to discern these minute molecular differences is driven by the resin's mixed mode functionality. The multimode functionality of PolyQUAT™ is obtained by covalently bonding PEI to the surface of highly cross linked polymethacrylate beads and functionalizing PEI to obtain tertiary and quaternary amine groups, thereby providing a primarily strong anion exchange with secondary weak anion exchange behavior.



Quaternary, tertiary and secondary provide anion exchange groups

## HIGH IONIC CAPACITY

Our BAKERBOND® line of polymeric resins were all designed with higher ionic capacities than the industry leading ion exchange and hydrophobic interaction chromatography (HIC) resins. The high ionic capacity increases the salt gradient needed to separate similar molecules, thus improving selectivity. This also enables operations at high salt concentrations. A comparison of ionic capacity between BAKERBOND® PolyQUAT™ and several other leading competitive hydrophobic interaction resins can be seen below.

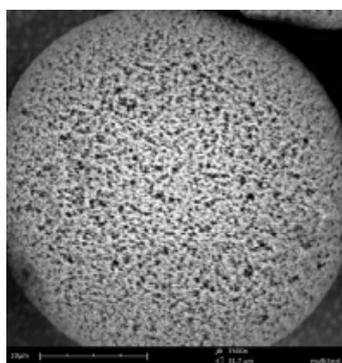


## EASE OF USE

Our BAKERBOND® line of polymeric resins are based on a highly cross linked rigid spherical polymethacrylate media that provides optimal porosity, high mechanical strength, and excellent chemical resistivity delivered with a consistent narrow particle size distribution. These features enable robust resin application, narrow elution bands for concentrated product fractions, and provide consistency and stability to downstream operations.

As illustrated in Fig.2, BAKERBOND® PolyQUAT™ has an average pore size of 500 Å for an exclusion limit of  $1 \times 10^6$  dalton. This open porosity coupled with the bead's mechanical strength allows for the use of PolyQUAT™ at high linear velocities due to good mass transfer.

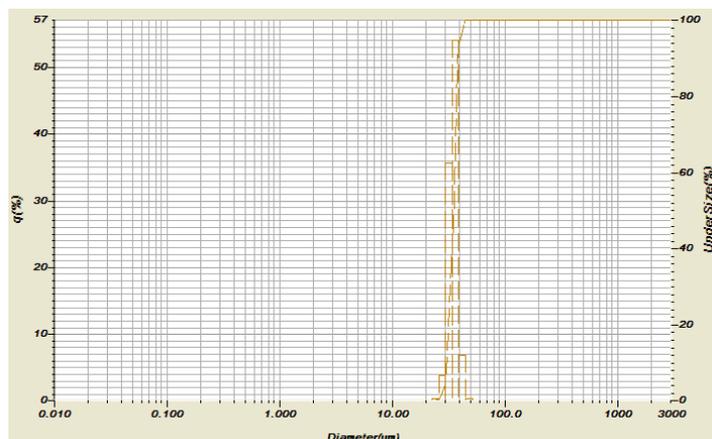
Fig.2. SEM image of polymethacrylate spherical beads at 2,000 magnification



The average pore size of the beads is 500 Å, providing an exclusion limit of  $1 \times 10^6$  dalton

A small average particle size of 35 µm combined with a narrow particle size distribution ( $d_{90}/d_{10}$ ) produces narrow elution bands of highly concentrated product for improved efficiency. This increases resolution and decreases pool volumes compared to media based on larger particles. High efficiency in combination with high selectivity enables higher loading levels while maintaining separation.

Fig.3. Particle size distribution of a typical lot of polymethacrylate beads



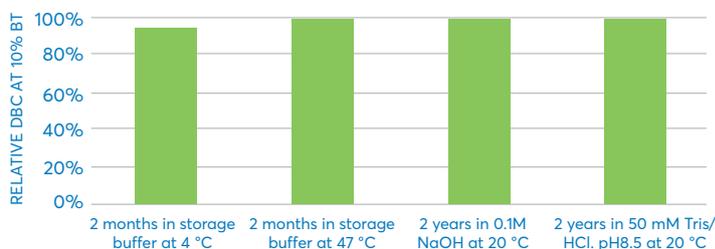
A median particle size of 35 micron and a particle size distribution of less than 1.4 ( $d_{90}/d_{10}$ ) show a lack of fines and result in high efficiency at low back pressure.

The hydrophilic backbone of BAKERBOND® PolyQUAT™ has low non-specific binding and the ability to withstand prolonged contact with commonly used cleaning and sanitizing solutions. PolyQUAT™ media can be easily packed to bed heights of up to 40 cm and operated in the linear velocity range of up to 500 cm/hr using conventional columns with pressure ratings of 2 to 10 bar. Our entire line of BAKERBOND® chromatography resins, buffers, and additive are manufactured under the strictest controls and is supported by world class quality systems and application resources.

## STABILITY AND CONSISTENCY

The performance of BAKERBOND® PolyQUAT™ remains consistent across a broad range of storage conditions. As illustrated in FIG.4 and FIG.5, little to no change was identified in protein binding capacity after prolonged storage across a wide range of pH's and temperatures. Product performance was evaluated by measuring the dynamic binding capacity of ovalbumin. Similarly, the purification behavior of PolyQUAT™ showed little to no change in its ability to separate a mixture of Cytochrome C, rabbit IgG, and β-lactoglobulin after subjecting the column to a comparable range of storage conditions.

Fig. 4. Relative dynamic binding capacity (DBC) of ovalbumin under various storage conditions



**Sample:** 4 mg/mL ovalbumin in binding buffer

**Column:** 10 × 100 mm

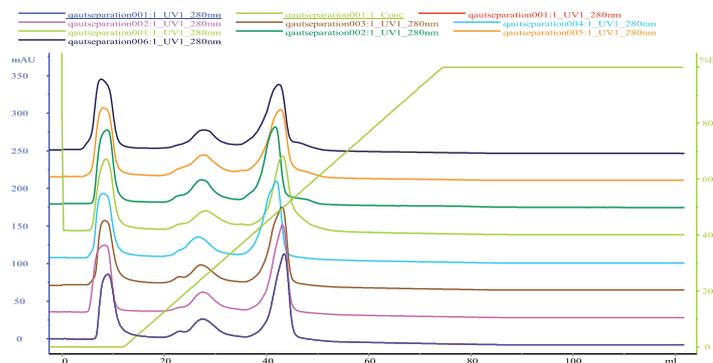
**Flow rate:** 5 mL/min.

**Binding Buffer:** 50 mM CAPS, pH 10.0

**Elution Buffer:** 1 M NaCl in binding buffer.



Fig. 5. Chemical stability of PolyQUAT



**Sample:** Cytochrome C, rabbit IgG and  $\beta$ -lactoglobulin

**Column:** 10 x 100 mm

**Flow rate:** 5 mL/min.

**Binding Buffer:** 50 mM TRIS, pH 8.5

**Elution Buffer:** binding buffer plus 1 M NaCl.

**Chromatograms from the top:**

1. PolyQUAT stored in storage solution for 2 years at 47 °C.
2. PolyQUAT stored in storage solution for 2 years at 4 °C.
3. PolyQUAT stored in 0.1 M NaOH for 2 years at 22 °C.
4. PolyQUAT stored in 50 mM H<sub>3</sub>PO<sub>4</sub> for 2 years at 22 °C.
5. PolyQUAT stored in 0.5 M NaOH for 2 months at 47 °C.
6. PolyQUAT stored in 50 mM H<sub>3</sub>PO<sub>4</sub> for 2 months at 47 °C.
7. PolyQUAT stored in storage solution for 2 months at 47 °C.
8. PolyQUAT stored in storage solution for 2 months at 4 °C.

**OPERATIONAL FLEXIBILITY**

BAKERBOND® PolyQUAT™ scales easily to the production environment with reproducible results in columns ranging from 20 to 45 cm in internal diameter. The narrow particle size distribution and mechanical stability of the media maintains conventional low pressure column performance at bed heights up to 20 cm. Modern medium pressure columns, rated at 7 bar, can be used to pack beds up to 40 cm in height and enable operation at high linear velocities.

The linear pressure-flow curve for BAKERBOND® PolyQUAT™ indicates that there is no resin compression at a bed height of 21.5 cm and a column diameter of 20 cm. In addition, the media does not need to be defined prior to the initial packing or subsequent packings, reducing the consumption of buffers and simplifying operations.

Fig.6. Pressure-Flow relation of polymethacrylate

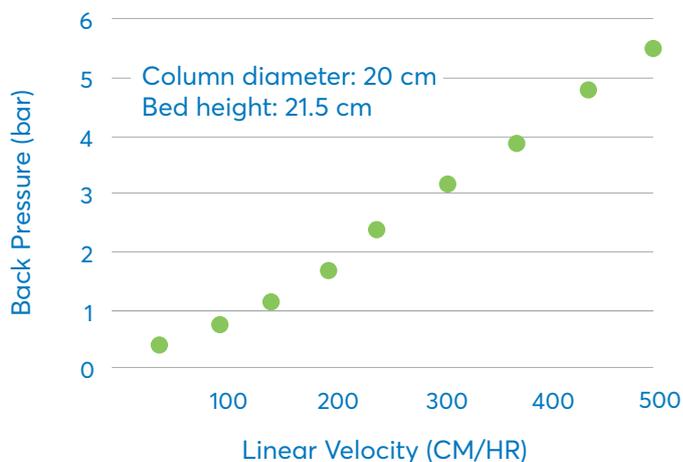


Fig.7. Efficiency of column (20 id x 21.5 cm high) packed with polymethacrylate based media

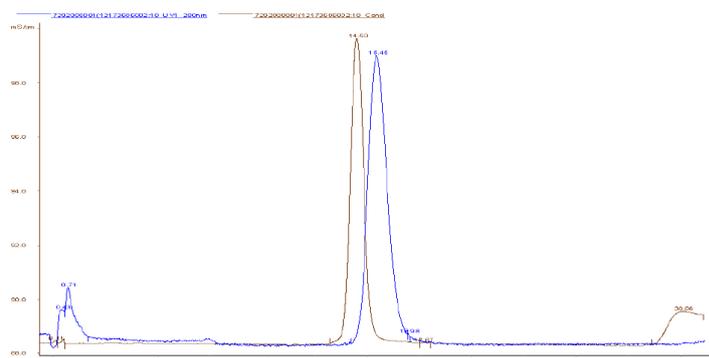


Fig.8. Scale-up to a BPG Column



## CONVENIENT PACKAGING

BAKERBOND® PolyQUAT™ resin comes conveniently packaged in a non-hazardous, non-flammable storage buffer, eliminating many of the burdensome shipping, handling, and storage requirements common among chromatography resins.

### Product ordering information

Size	Product Number
1 mL Lab Columns (5-pack)	6065-07
5 mL Lab Columns (5-pack)	6065-25
50 mL	7603-01
100 mL	7603-02
500 mL	7603-03
1 L	7603-04
5 L	7603-05

## PROCESS AND APPLICATION SUPPORT

Avantor has deep expertise in process chromatography optimization and can work with you to help ensure that BAKERBOND® PolyQUAT™ delivers the improved performance you need in downstream processing. Technical support from our scientists and application specialists is available from our multiple global research and innovation centers.

### Product Information Summary

#### Characteristics

Functionality	Primarily a strong anion exchanger with weak anion exchange sites
Functional Group	-NH-CH <sub>2</sub> -CH(OH)-CH <sub>2</sub> -N+(CH <sub>3</sub> ) <sub>3</sub>
Ion Exchange Capacity	0.4 – 0.6 Cl meq/mL
Median Particle Size	35 µm
Particle Size Distribution (d <sub>60</sub> /d <sub>10</sub> )	< 1.4
Median Pore Size	500 Å
Exclusion Limit	1 × 10 <sup>6</sup> Daltons
Operating pH Range	4.5 – 14.0
Cleaning pH Range	1.0 – 14.0
Chemical Stability	All commonly used aqueous buffers, sodium hydroxide, acetic acid, phosphoric acid, guanidine hydrochloride, up to 100% ethanol, methanol, or 2-Propanol.
Shipping Solvent	Media is shipped as 1:1 slurry in acetate buffer containing benzyl alcohol at pH 4.5 and can be stored for up to 5 years at 4–15 °C. The media can also be stored in 20% ethanol or 0.1 M NaOH for up to 5 years.



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