J.T.Baker BAKERBOND Polymeric Multimode Ion Exchange Chromatography Media

Ion exchange chromatography is one of the most widely used methods in purification of biopharmaceutical drug such as recombinant protein, monoclonal antibody, antibody related product, and DNA.

This documents contains technical information of BAKERBOND™ PolyIEX media and column packing procedure.

BAKERBOND™ PolyIEX media are based on spherical polymethacrylate beads covalently bonded to polyethylenimine and additional ionic groups to offer primary anion or cation functionalities along with secondary anion exchange sites.

This unique design results in multimodal behavior and offers enhanced selectivity and high efficiency when compared to conventional ion exchange media. PolyIEX is alkaline stable and its operational flexibility makes it ideal for use in various chromatographic purifications, where PolyIEX can be packed with relative ease. PolyIEX offers the following attributes to provide the highest resolution and productivity:

- Unique selectivity
- High efficiency
- Scalable and robustness
- Manufactured in GMP with appropriate quality system
- Global application support

Description	Cat No.
Poly PEI	7585
Poly Quat	7603
Poly ABx	7586
Poly CSx	7587

Information

Property	PolyPEI	PolyABx	PolyCSx	PolyQUAT
Functionality	Primary weak anion exchanger + hydrophilicity	Multimode Primary weak cation exchanger with weak anion exchange sites+ hydrophilicity	Multimode Primary strong cation exchanger with weak cation and anion exchange sites + hydrophilicity	Multimode Primary strong anion exchanger with weak anion exchange sites + hydrophilicity
Functional Group	CH2CH2NH	–NH–C(=0)–CH2–CH2-COOH	–NH–C(=0)–CH(SO3H)–CH2–COOH	-NH-CH2-CH(OH)-CH2-N+(CH3)3
Ionic Capacity	0.3–0.5 meq/mL as chloride	0.15–0.25 mmol H+/mL (dynamic)	0.3-0.5 meq/mL	0.4-0.6 meq/mL
Average Particle Size	35 µm			
Median Pore Diameter	500 Å			
Porosity	4 x 106 (globular proteins)			
Operating pH Range	4.5–9	4.5-14	4.5-14	4.5-14
Cleaning (CIP)	50 mM Phosphoric acid ; 0.1-1.0 N NaOH; pH range: 1.0-14			
Chemical Stability	All commonly used aqueous buffers, sodium hydroxide, acetic acid, phosphoric acid, urea, guanidine hydrochloride, up to 100% ethanol, methanol, or isopropanol			

Stability

	PolyPEI	PolyABx	PolyCSx	PolyQUAT
Storage Stability of Media / Column				
Storage Buffer*	5 yr	5 yr	5 yr	5 yr
H_3PO_4 50 mM	5 yr	5 yr	5 yr	5 yr
NaOH 0.1 M	5 yr	5 yr	5 yr	5 yr
Clean-in-Place Stability of Media / Column				
H_3PO_4 50 mM	> 48 h	> 48 h	> 48 h	> 48 h
NaOH 0.1 M	> 48 h	> 48 h	> 48 h	> 48 h
NaOH 0.5 M	> 2 h 0.5 h, 80°C	> 2 h	> 2 h	> 2 h 0.5 h, 80°C
Reuse Stability				
Column Pack/Unpack	> 100			

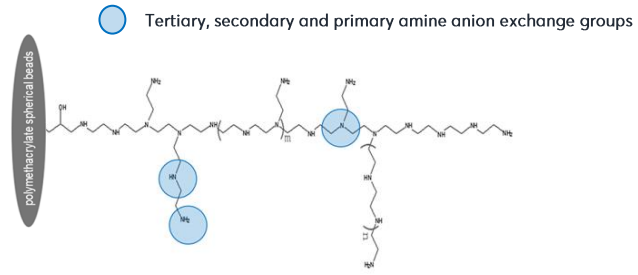
- Media are also stable in ethanol or GuHCl
- Stability is indicated by capacity, separation and particle size

* Storage buffer : 200 mM Sodium acetate, 2% benzyl alcohol, pH 4.2

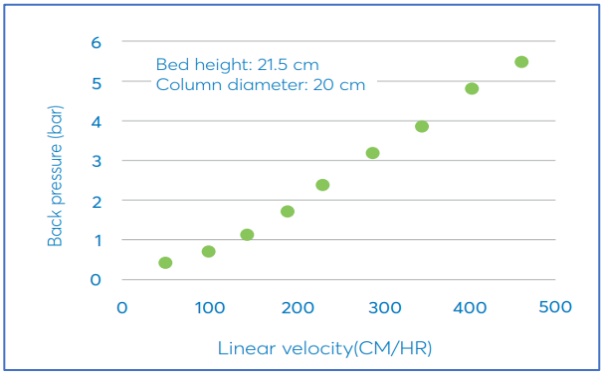
JT. Baker BAKERBOND Poly PEI

Weak anion exchanger chromatography media

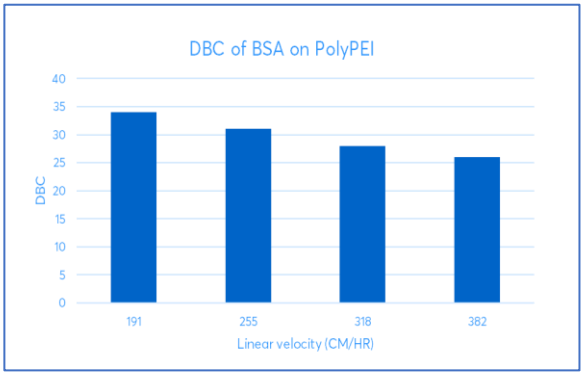
BAKERBOND PolyPEI is a multimode ion exchanger functioning as a weak anion exchanger over a wide pH range. The unique selectivity of PolyPEI is the result of proprietary surface chemistry with weak anionic exchange sites due to the presence of primary, secondary and tertiary nitrogen on the Polyethyleneimine (PEI) ligands. The presence of amino groups with different pKa offers better selectivity than conventional weak anion exchange media with equivalent capacities, and it is capable of separating proteins and peptides having similar isoelectric points(pI). Unique selectivity of closely related molecules is often achieved with PolyPEI where conventional ion exchangers fail to provide sufficient separation in a process environment.



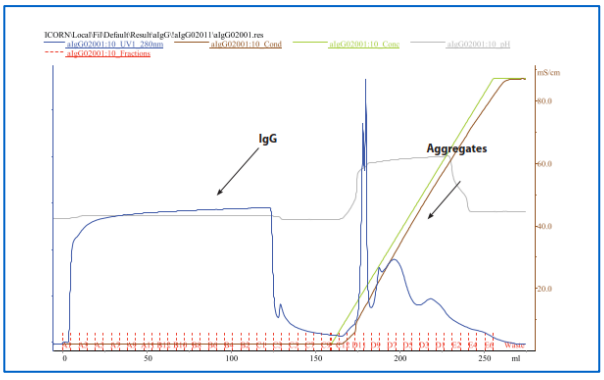
Property	PolyPEI
Functionality	Primary weak anion exchanger + hydrophilicity
Functional Group	CH2CH2NH
Ionic Capacity	0.3–0.5 meq/mL as chloride



The linear behavior of the pressure – flow curve indicates that there is no resin compression.



Dynamic Binding Capacity of BSA on PolyPEI at High Linear Velocity



IgG Flow-through mode application

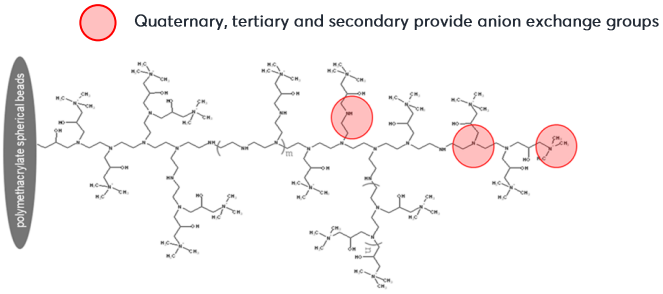
Mode : Flow-through application
Sample : hIgG
Resin : BAKERBOND PolyPEI
EQ Buffer : 50 mM MES pH 6.5
Elution Buffer : 50 mM MES, 1 M NaCl, pH 6.5
Linear gradient from 0 to 100% B in 10 CV
Flow rate: 1.2 mL/minute

Flow-through mode with PEI is applied for hIgG purification.
Aggregate form and other impurities are removed during this purification. Purified IgG is collected by F/T.

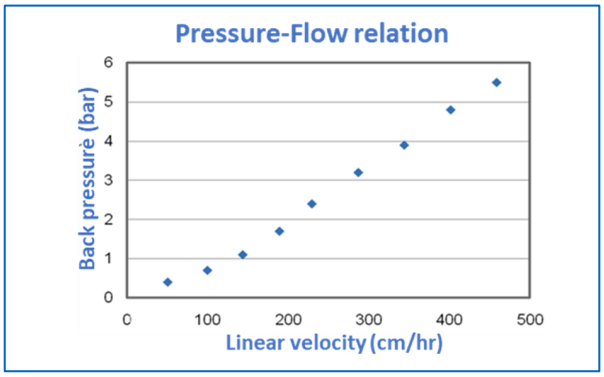
J. T. Baker Poly Quat

Strong anion exchanger chromatography media

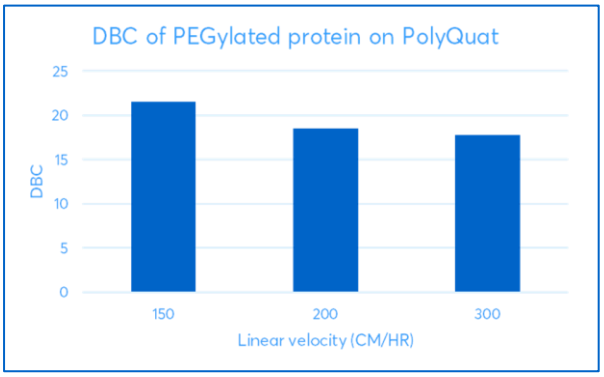
BAKERBOND PolyQuat is a multimode ion exchanger functioning as a strong anion exchanger over a wide pH range. Secondary anion exchange sites are due to the presence of amine groups. This multimodal functionality offers better selectivity than conventional strong anion exchange media with equivalent capacities and is capable of separating proteins and peptides having similar isoelectric points (pI). Unique selectivity of closely related molecules is often achieved with PolyQUAT where conventional ion exchangers fail to provide sufficient separation in a process environment.



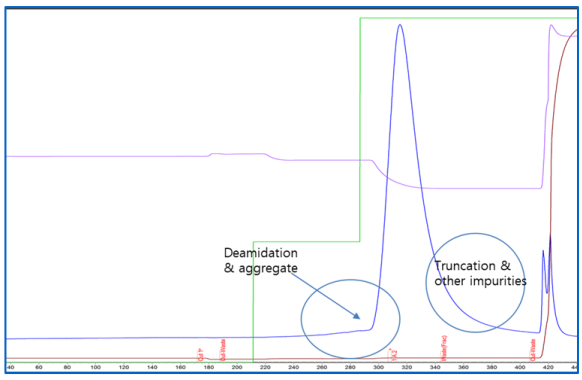
Property	PolyQuat
Functionality	Multimode Primary strong anion exchanger with weak anion exchange sites + hydrophilicity
Functional Group	-NH-CH2-CH(OH)-CH2-N+(CH3)3
Ionic Capacity	0.4-0.6 meq/mL



The linear behavior of the pressure – flow curve indicates that there is no resin compression.



Dynamic Binding Capacity of BSA on PolyQuat High Linear Velocity



Protein polishing step application

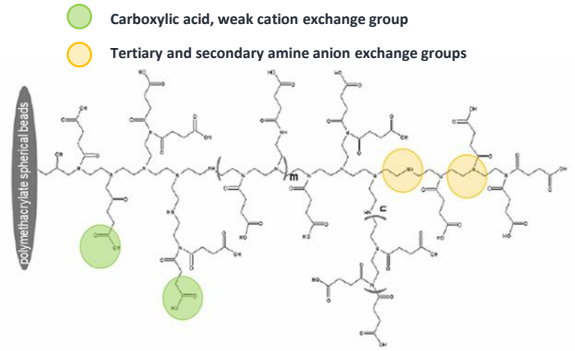
Resin : BAKERBOND PolyQuat
Sample: rProtein Polishing step application
EQ Buffer : 50 mM Sodium phosphate, pH 8.0
Elution Buffer: 65 mM Phospho-citrate, pH 5.8

PolyQuat is applied for removing deamidation, truncation and other form of impurity.
Peak is separated 3 parts which are main target drug peak and other 2 impurity peaks.
Front of main peak contains deamidation and aggregate.
Tail of main peak contains truncation and other impurities.

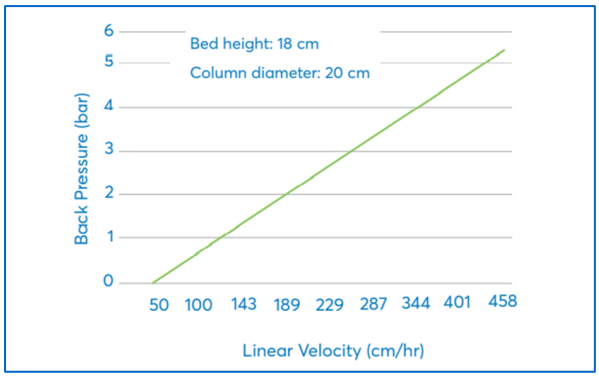
J. T. Baker BAKERBOND Poly ABx

Weak cation exchanger chromatography media

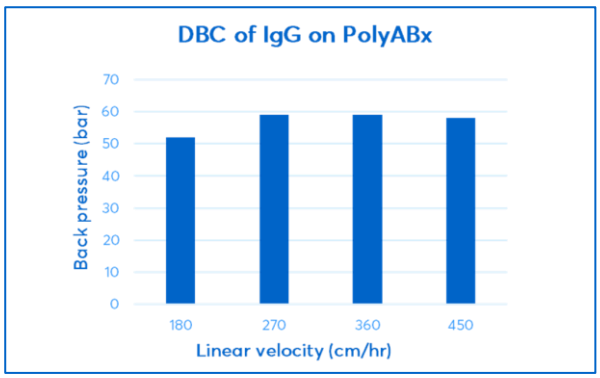
BAKERBOND ABx is a multimode ion exchanger which primarily functions as a weak cation exchanger over a wide pH range. Secondary anion exchange sites are due to the presence of amine groups. This multimodal functionality offers better selectivity than conventional weak cation exchange media with equivalent capacities and is capable of separating proteins and peptides having similar isoelectric points (pI). Unique selectivity of closely related molecules is often achieved with PolyABx where conventional ion exchangers fail to provide sufficient separation in a process environment.



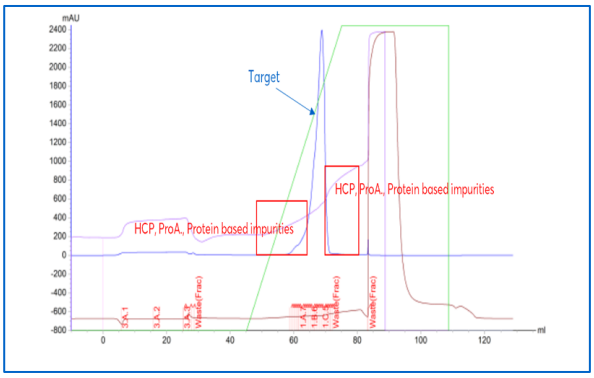
Property	PolyABx
Functionality	Multimode Primary weak cation exchanger with weak anion exchange sites + hydrophilicity
Functional Group	–NH–C(=0)–CH2–CH2-COOH
Ionic Capacity	0.15–0.25 mmol H+/mL (dynamic)



The linear behavior of the pressure – flow curve indicates that there is no resin compression.



Dynamic Binding Capacity of BSA on PolyABx High Linear Velocity



FC fusion protein polishing step application

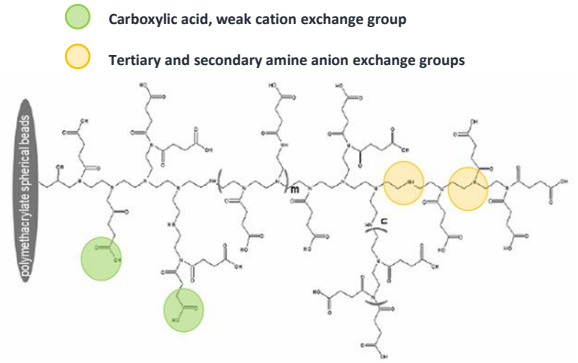
Resin: BAKERBOND PolyABx
Sample: Fc fusion protein polishing step application
EQ Buffer: 50 mM Phospho-citrate, 5 mM Sodium chloride pH 5.0
Elution Buffer: 50 mM Sodium phosphate, pH 8.0

PolyABx is applied in polishing step of Fc fusion protein purification.
HCP, residual protein A and other impurities are removed at this step.
In residual protein A , the input contains 15.2 ppm of residual protein A, but the output contains 1.1 ppm
(> 90 % of reduction rate).

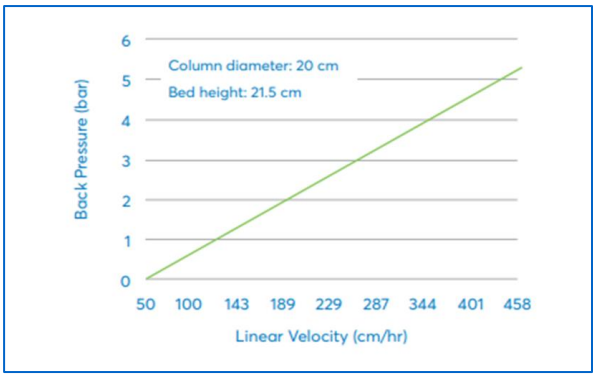
J. T. Baker BAKERBOND Poly CSx

Strong cation exchanger chromatography media

BAKERBOND CSx is a multimode ion exchanger functioning as a strong cation exchanger over a wide pH range. Its strong cationic exchange stites are a result of sulfonic acid group. Weak cationic exchange sites are due to carboxylic acid groups, while weak anion exchange behavior is maintained by the presence of amino groups on the Polyethyleneimine (PEI) ligands. This multimodal functionality offers better selectivity than conventional strong cation exchange media with equivalent capacities and is capable of separating proteins and peptides having similar isoelectric points (pI). Unique selectivity of closely related molecules is often achieved with PolyCSx where conventional ion exchangers fail to provide sufficient separation in a process environment.



Property	PolyABx
Functionality	Multimode Primary weak cation exchanger with weak anion exchange sites + hydrophilicity
Functional Group	–NH–C(=0)–CH2–CH2-COOH
Ionic Capacity	0.15–0.25 mmol H+/mL (dynamic)



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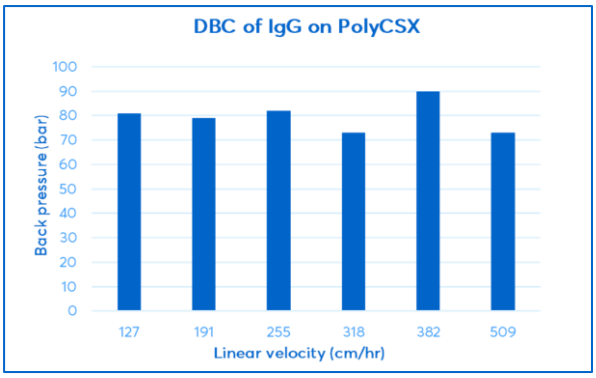
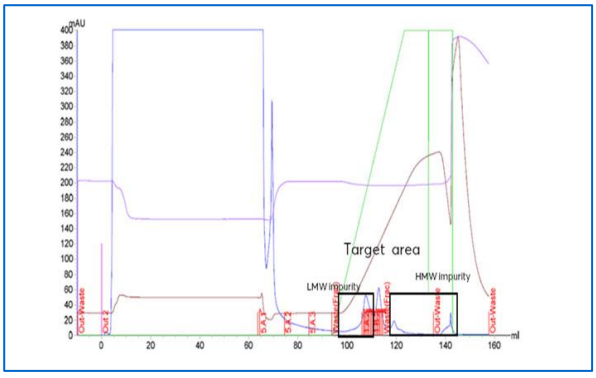


Dynamic Binding Capacity of BSA on PolyCSx High Linear Velocity



Fab capture step application

Resin : BAKERBOND PolyCSx
Sample: Fab capture step application
EQ Buffer : 50 mM Phospho-citrate, 50 mM Sodium chloride pH 6.5
Elution Buffer: 50 mM Phospho-citrate, 500 mM Sodium chloride, pH 6.5

Poly CSx is applied in capture step for Fab purification.
The input has 15.70 % purity, but the output has 79.44 % purities in SE-HPLC.

Column packing instruction

Formula

- Compression factor = $\frac{\text{Setled bed height}}{\text{Packed bed height}}$
- Column volume = column bed height (cm) x cross sectional area (cm^2)
- Settled media volume = column volumn x compression factor
- Slurry volume = $\frac{\text{Settled media volume}}{\text{Slurry concentration (\%)}}$

Packing guidance

The mobile phase, beforehand, needs to be determined in order to do the column packing on the ÄKTA™ system. The solvent can be determined as described in the table below.

1. Assemble the coloumn in accordance with instruction.
2. Connect the column to the stand holder or FPLC holder.
3. Mark the target height on the column.
4. Fill the column with around 1 cm packing buffer.
5. Load the column with calculated resin slurry.
6. Start down-flow into column.
7. Follow step-wise flow until delta-pressure reaches 90% of max pressure and check bed consolidation.
8. Calculate the final bed height.
9. The column is ready for the evaluation.

Evaluation

	Conductivity	UV280
Evaluation sample	0.8 M Sodium chloride	1% Acetone solution
Evaluation solution	0.4 M Sodium chloride	Water
Injection volume	1% of column volume	1 ~ 5 %
Flow rate	50 cm/hr	50 cm/hr

HETP and As are calculated from curve as below.

HETP = $\frac{L}{N}$

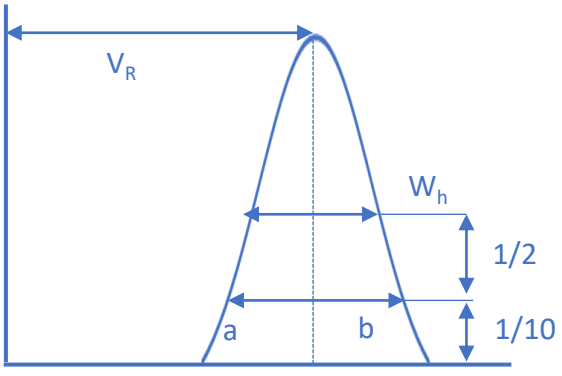
N = 5.54 x $(\frac{V_R}{W_h})^2$

The reduced plate height is described h and calculated as below. Less than 5 means high efficiency.

h = $\frac{HETP}{d_{50v}}$

Asymmetry = $\frac{b}{a}$

The peak has to be symmetrical, and the asymmetry factor has to be as close to 1 as possible and acceptance criteria are from 0.8 to 1.8.



L = bed height
N = Number of theoretical plate
V_R = Max peak height volume
W_h = Peak width of 50% points
d_{50v} = Median particle size (cm)
A = front peak width at 10% points
B = Tail peak width at 10% points

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