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Technical note

J.T.Baker® BAKERBOND® PROchievA™ recombinant protein A lab columns

J.T.Baker®

How to use the PROchievATM pre-packaged lab columns

(a) 1 mL PROchievA™ column

(b) 5 mL PROchievA™ column

PROTOCOL FOR PURIFICATION OF ANTIBODIES AND FC FUSION PROTEINS

This protocol provides typical operating conditions for purification of antibodies from a clarified cell culture. It has been developed based on experimental data for few representative molecules and can be modified based on sample type and antibody properties to optimize yield and purity. The optimization may involve changing buffer type, salt concentration, pH, wash buffer, and elution buffer.

COLUMN SPECIFICATIONS

- The material of construction of column is biocompatible polypropylene.
- The column hardware pressure limit is 5 bars.

Measurements:

- **1 mL BAKERBOND® PROchievA™ column:** 0.7 x 2.6 cm
- **5 mL BAKERBOND® PROchievA ™ column:** 1.6 x 2.6 cm

RESIN

The columns are packed with J.T.Baker® BAKERBOND® PROchievA™ recombinant protein A resin suitable for purification of monoclonal antibodies, FC fusion proteins and bispecific antibodies. The BAKERBOND® PROchievA™ protein A ligand is recombinant protein A and binds to Fc regions of IgG molecules. It is engineered to tolerate alkaline clean-in-place solutions comprised of 1M sodium hydroxide with additives such as sucrose, ethylene glycol, or propylene glycol. The ligand properties are optimized and attached to crosslinked agarose beads having average particle size of 70-80 µm using bifunctional linkers to maximize dynamic binding capacity.

BUFFERS

- **Equilibration buffer, wash buffer:** 10 mM sodium phosphate dibasic, 2 mM sodium phosphate monobasic, 137 mM sodium chloride, pH 7.4 (1X PBS)
- **Elution buffer:** 0.1 M Acetic acid or 0.1 M Citric acid, pH 3.0 – 3.8
- **Neutralization buffer:** 1.0 M Tris, pH 9.0
- **Cleaning Solution:** 0.1 M sodium hydroxide
- **Storage buffer:** shipped in 0.2 M sodium acetate, 2% ethanol, 2% benzyl alcohol, pH 5.5; After use, the column is recommended to be stored in the storage buffer. If needed, the columns can be also stored in 20% ethanol

COLUMN OPERATION SEQUENCE

Note: All recommended flow rates for steps 3 - 10 can be found in Table I.

PREPARATION

01. Remove the bottom stop plug from the column while keeping top end plug in place and attach the column to the instrument column outlet line.
02. Remove the top stop plug from the column and attach the column to instrument column inlet line while slowly flowing equilibration buffer to create a "wet" connection to avoid introducing air to the column.
03. If column is being used for the first time, flush the column with 2-5 column volume (CV) of water followed by 2-5 CV of cleaning solution at equilibrium flow rate.

PURIFICATION

04. Equilibrate the column with 3-10 CV of equilibration buffer. If pH of outlet from column reaches to +/- 0.1 unit of equilibration buffer, column is considered equilibrated.
05. Apply the sample to the column at the desired flow rate, listed in Table I within the column dynamic binding capacity. Typical dynamic binding capacity at corresponding residence times can be found in Table I. The dynamic binding capacity may vary (+/- 20%) depending on type of molecule and binding conditions.
06. Wash the column with 5 - 10 CV of wash buffer or until no material is present in eluent.
07. Elute the target material from the column using 2 - 5 CV of elution buffer. Collect elution into tubes containing 100 µL of 1 M Tris, pH 9.0 per 1.0 mL of elution.
08. Equilibrate the column with 3-10 CV of equilibration buffer.

CIP AND STORAGE

09. If cleaning the column, flow minimum 5CV of cleaning solution over the column at a flow rate that ensures a 15-minute contact time of the alkaline solution with the column. This step can be performed after every cycle or every campaign of desired runs – for example, every 5 runs – depending on the sample.
10. If using the column again, proceed to step 4. If storing the column, proceed to step 11.
11. Flow 5 - 10 CV of storage buffer over the column. Ensure pH of column outlet reaches +/- 0.1 unit of storage buffer pH. Column can then be detached from purification instrument. Attach top and bottom stop plugs to the column. Store the column at 2 – 8^{0}C until next use.

Table I: Typical protocol conditions

Purification protocol step	Step	Recommend buffer usage column volumes	1 mL column recommended flow rate*	5 mL column recommended flow rate*
4	Equilibration	3 - 10	1.00 mL/min (157cm/h)	5.00 mL/min(157cm/h)
5	Load	TBD by User	TBD by User	TBD by User
			2 min RT – 0.50 mL/min	2 min RT – 2.50 mL/min
			4 min RT – 0.25 mL/min	4 min RT – 1.25 mL/min
			6 min RT – 0.17 mL/min	6 min RT – 0.83 mL/min
			8 min RT – 0.13 mL/min	8 min RT – 0.63 mL/min
			Typical expected dynamic binding capacities mg/ml.	
			2 min RT - 35 mg/mL	
			4 min RT - 50 mg/mL	
			6 min RT - 60 mg/mL	
			8 min RT - 65 mg/mL	
6	Wash	5 – 10	1.00 mL/min	5.00 mL/min
7	Elute	2 - 5	1.00 mL/min	5.00 mL/min
8	Equilibration	3 – 10	1.00 mL/min	5.00 mL/min
9	Cleaning	15-min contact time with minimum 5CV	0.5 - 1.0mL/min	1.0 - 5.0mL/min
11	Storage	5 - 10	1.00 mL/min	5.00 mL/min

*mL/min is same as CV/min for these columns

Table II: Alternative buffer and operational pH range of each step

Step	Standard	Alternative buffer	Standard pH	pH range
Equilibration, load	1X PBS	HEPES, Tris buffer	7.4	7.0-9.0
Wash	1X PBS	HEPES, Tris buffer with additives such as arginine, propylene glycol, IPA, Urea, Guanidine HCl or polysorbate 20	7.4	7.0-10.0
Elute	0.1 M Acetic acid or 0.1 M Citric acid	Acid with up to 1 M NaCl	3.4	3.0-3.8
Cleaning	0.1M NaOH	0.5 M or 1.0 M NaOH* or 0.5 M or 1.0 M NaOH with either 1M sucrose, 20 % ethylene glycol, or 20% propylene glycol**	-	-

*** 0.5 and 1.0 M NaOH can be used to effectively clean the resin at 2-8°C.**

****The addition of sucrose, ethylene glycol, or propylene glycol to these solutions can be used to help preserve column lifetime when cleaning is performed at ambient conditions.**

Table III: Recommended buffers, salt and cleaning solutions

Recommended product	Avantor Part Number	VWR Part Number – North America	VWR Part Number – Europe & Asia	Grade*	Manufacturing quality standard
Sodium phosphate dibasic anhydrous	3826	JT3826	3826	USP, Endotoxin tested	GMP
Sodium phosphate monobasic monohydrate	3802	JT3802	3802	Multicompendial	GMP
Sodium chloride	3625	JT3625	3625	Multicompendial	GMP
Acetic acid	9526	JT9526	9526	Multicompendial	GMP
Citric acid	127	JT0127	127	Multicompendial	GMP
Tris (base)	4102	JT4102	4102	Multicompendial	GMP
Trishcl	4106	JT4106	4106	Biotech Reagent	GMP
Sodium acetate	3474	JT3474	3474	USP, FCC	GMP
Ethanol denatured 70%	P004	JTP004	P004	BAKER	GMP
Benzyl alcohol	9039	JT9039	9039	Multicompendial	GMP
1.0N NaOH	328	JT0328	328	Biotech Reagent	GMP
0.5N NaOH	329	JT0329	329	Biotech Reagent	GMP
0.1N NaOH	5636	JT5636	5636	BAKER-ANALYZED	-
Hepes sodium salt	4153	JT4153	4153	Ultrapure Bioreagent Molecular biology grade	-
L-arginine	2066	JT 2066	2066	Multicompendial	-
Propylene glycol	9403	JT9403	9403	Multicompendial	GMP
Urea	4203	JT4203	4203	Multicompendial	GMP
Guanidine hcl	510	JT0510	510	Biotech Reagent	GMP
Polysorbate 20	4116	JT4116	4116	Multicompendial	GMP
D-(+)-Sucrose, crystals	4074	JT4074	4074	Multicompendial	GMP
Ethylene glycol, 99%	5387	JT5387	5387	Baker	-
(±)-1,2 Propanediol ≥ 99.5%	9403	JT9403	9403	Multicompendial	GMP

*Multicompendial - meets USP, EP, BP, JP and ChP compendia as applicable.

*Detailed product information is available at **www.avantorsciences.com** and **www.vwr.com**, or for more information contact your Avantor account manager.

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