



AcroSep™ Protein A Columns

- Pre-packed columns for affinity-based protein purification.
- Patented HyperD® “gel-in-a-shell” resin offers a very stable Protein A linkage.
- Versatile use:
 - Fully automated in combination with an automated chromatography instrument such as the ÄKTAdesign* systems.
 - Semi-automated in combination with pumps.
 - Manual use in combination with a syringe.

Ordering Information

<u>Part Number</u>	<u>Description</u>	<u>Color</u>	<u>Column Volume</u>	<u>Pkg</u>
20078-C001	Protein A Ceramic HyperD F	Pearl	1 mL	5/pkg

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Note: *The procedures herein are intended only as a guide. Users should always verify product performance with their specific applications under actual use conditions. If you have questions about the information presented in this guide, please contact Pall Life Sciences technical service.*

Specifications

Materials of Construction

Column Housing, Cap, Plug, and Adapter: Polypropylene
Column Frit: Polyethylene

Protein A Ceramic HyperD Properties

Particle Size: 50 μm (average)
DBC for Human IgG @ 10% Breakthrough: $\geq 25 \text{ mg/mL}^{\dagger}$
Ligand: Recombinant Protein A
Immobilized Protein A/mL of Resin: 3-5 mg/mL
Operation pH Range: 2-11
Cleaning pH: 2-13

Column Geometry

Column Volume: 1.04 mL
Bed Height: 1.48 cm (0.58 in.)
Bed Diameter: 0.94 cm (0.37 in.)

Device Dimensions

Diameter: 1.6 cm (0.6 in.)
Length (Without Plugs): 4.8 cm (1.9 in.)

Connections

Inlet: Threaded female luer
Outlet: Rotating male luer locking hub

Flow Rates

Recommended flow rate 0.2-1.0 mL/min; residence time (T_r) = 5.2-1.04 min

Maximum Column Pressure

3 bar (300 kPa, 43.5 psi)

Storage

2-8 $^{\circ}\text{C}$ (36-46 $^{\circ}\text{F}$); do not freeze

[†]Determined using 10 mg/mL human IgG in PBS, pH 7.4; elution with 0.1 M sodium citrate, pH 2.5; column: 4.6 mm ID x 100 mm; flow rate: 100 cm/hr; T_r = 5.9 min.

Note: DBC for Human IgG varies with IgG source.

Working Conditions

In most cases, sample should be loaded onto the column in a buffer at a neutral pH and physiological ionic strength (i.e., PBS, pH 7.4). Once eluted, the antibody solution must be neutralized immediately to limit aggregation. This is best accomplished with the addition of a small amount of high concentration buffer (e.g., 1M Tris pH 8-8.5) into the tubes for elute collection prior to the purification run. The affinity of Protein A for a particular antibody is dependent on species and isotype. These affinities are well characterized and published. The capacity of Protein A Ceramic HyperD F resin is also influenced by the specific purification conditions. Additionally, different sources of pure antibody of the same species and isotype may show varied DBC.

The following conditions/buffers are recommended for specific types of antibodies:

Human IgG

- Load: 10-25 mM phosphate (or Tris or PBS), pH 7.4-8.0
- Wash: 10-25 mM phosphate + 500 mM NaCl (or PBS), pH 7.4-8.0
- Elute: 100 mM sodium citrate, pH 2.5

Murine Mabs

Mouse IgG_{2a}, IgG_{2b}, IgG₃:

- Load: 10-25 mM phosphate (or Tris), pH 7.4-8.0
- Wash: 10-25 mM phosphate + 500 mM NaCl, pH 7.4-8.0
- Elute: 100 mM sodium citrate, pH 2.5-5.0
 - For IgG_{2a}: Choose an elution pH between 4 and 5
 - For IgG_{2b} and IgG₃: Choose an elution pH between 3 and 4

Working Conditions *(continued)***Mouse IgG₁**

The affinity of IgG₁ is weak, thus, best capacity requires altered conditions.

- Load and wash: 1.5 M glycine and 3 M NaCl, pH 8.9
- Elute: 100 mM sodium citrate, pH 4-6

Notes:

- *Elution pH between 4.5-4.7 allows for direct injection of the purified eluted antibody fraction onto a CM Ceramic HyperD F column (PN 20050-C001).*
- *For purification of mouse IgG from ascites fluid, dilute the sample 1:4 dilution in loading buffer.*
- *For initial trials, a working flow rate of 100 cm/hr is recommended. Antibody binding capacity is increased significantly when lower flow rates are used for load and/or wash steps. If necessary, flow rate can be increased for stripping and regeneration steps.*

**Influence of Flow Rate and Residence Time on Binding Capacity
(at 10% Breakthrough)**

<u>Flow Rate</u>	<u>Residence Time</u>	<u>Average DBC (mg protein/mL resin)</u>	<u>Std Dev</u>
0.2 mL/min	5.2 min	35.8	3.27
1 mL/min	1.04 min	27.4	1.24
4 mL/min	0.26 min	19.5	0.48

Residence Time Calculation:

$$Tr = (CSA \times ht) / FR$$

Tr - Residence Time (min)

CSA - Cross Sectional Area = πr^2 (cm²)

Ht - Bed Height (cm)

FR - Flow Rate (mL/min or cm³/min)

Using the AcroSep 1 mL pre-packed column geometry in this calculation, Tr = 1.036 cm³/flow rate (mL/min). Affinity interactions are generally very sensitive to residence time, and this is particularly noted with Protein A, as demonstrated by the data above. Lower flow rates are recommended for maximum binding capacity, especially when antibody is very dilute.

Instructions for Use – Automated or Pumped Chromatography Systems

Materials Required

- System (ÄKTAdesign System, pump, or equivalent)
- Filtered and degassed buffers

Automated System Protocol

1. Attach column to pre-primed system. To prevent air from getting into the column, fill the neck of the column dropwise while system is running very slowly. Allow the buffer to flow through the column until all bubbles in the bottom of the column have been evacuated.
2. Wash column with 5 column volumes (CV) of loading buffer.
3. Wash column with 5 CV of elution buffer.
4. Equilibrate with 5-10 CV of running buffer.
5. Load the sample.
6. Wash with at least 5 CV of loading buffer or until the OD_{280} reading returns to baseline level.
7. Elute with chosen elution buffer, stepwise, or gradient. (See Elution under Purification Optimization, page 7)
8. Strip with 5-10 CV of elution buffer.
9. Re-equilibrate with 5 CV loading buffer.

Instructions for Use – Manual Use with Syringe

Materials Required

- Syringes with luer lock fittings
- Filtered buffers

Syringe Protocol

Note: *It is important to avoid introducing air into the column. Remove air bubbles from fluid filled syringe before attachment to the column each time the syringe is changed.*

When pushing fluid through the syringe, maintain a relatively constant flow rate with minimal backpressure, typically 0.5-1 mL/min. A syringe pump can be used if available.

1. Fill a syringe with loading buffer. To avoid getting air into the column, load syringe with more than the required amount of buffer.
2. Equilibrate the column with 5-10 CV of loading buffer by securing the filled syringe to the column luer connector. Check that there are no air bubbles at the site of attachment then apply gentle pressure to push the buffer through the column.
3. Fill a syringe with sample.
4. Load sample onto the column avoiding the introduction of air bubbles.
 - Collect all flow through sample to assess possible IgG breakthrough if capacity is exceeded or flow rate is too high.
5. Wash column with 5 CV of loading buffer to remove unbound proteins.
 - Collect flow through sample for analysis if desired.
6. Fill syringe with elution buffer. Secure the filled syringe to the column luer connector. Check that there are no air bubbles at the site of attachment.
7. Run 10-20 CV of elution buffer through the column to elute bound antibodies.
 - Collect all flow through sample containing eluted protein(s) in appropriated sized fractions.
 - Tubes for eluates should contain a neutralization buffer prior to antibody elution.
8. If the column will be reused, strip residual protein with 5 CV of low pH buffer.
9. Fill the syringe with loading buffer. Equilibrate the column with 5-10 CV of loading buffer.

Purification Optimization

All buffers that contact the antibody must be known to maintain antibody solubility for all antibodies in the sample at the time of exposure to those buffers. If there are any doubts, this should be tested in advance to avoid protein loss and fouling of the column.

Method Optimization Analysis

- Analyze elute fractions to ensure that target antibody(s) is captured under the chosen conditions and binding capacity for target antibody(s) is not exceeded.
- If the antibody is not efficiently captured, reduce the flow rate during the binding/loading step.
- Analyze all elute fractions to determine which contain the highest levels of purity and activity of target molecule (for example by ELISA, OD₂₈₀, SDS-PAGE, or other appropriate techniques).

Elution

- Protein A elution buffer is generally a low pH buffer that will not impact the stability of the antibody during short term exposure. Thus, elution pH should be increased somewhat for acid sensitive antibodies.
- All eluted antibodies should be neutralized as quickly as possible after elution in order to maintain the antibody structure and function, and prevent antibody aggregation and/or precipitation.
- Linear gradient
 - If the exact elution point of the target molecule is unknown or more than one antibody (species or isotype) or antibody structure (aggregated vs. monomeric) is to be purified, a linear gradient can be used to try and separate the target and undesired antibodies.
 - A longer gradient with a shallow slope (more volume used for concentration change) will result in better separation of antibody species in a larger volume (broad peaks). A shorter, steeper slope will result in sharper peaks with less separation.

Purification Optimization (*continued*)

- Step elution
 - Stepwise elution can be used to simplify a purification process. However, it is important to know the elution behavior of the target and impurities before designing this procedure. This type of elution typically takes less time than a gradient elution.
- OPTIONAL:** For the highest degree of purity, a common step protocol has four post-antibody capture steps.
 1. Once the antibody capture (loading step) is complete, the column is washed with elution buffer with a lower pH than the loading buffer, but not low enough to elute the target antibody. Wash to baseline OD₂₈₀, typically 5 CV. This step is only recommended if unwanted antibody is in the starting sample.
 2. Then the target antibody is eluted with a further decrease in pH. pH is lowered just enough to elute the target antibody and as few other contaminants as possible. Elute to baseline.
 3. If more than one protein is to be purified, a second elution step can be added before the low pH column strip.
 4. Finally the lowest pH is used to strip the remaining proteins from the column. Wash to baseline. This is only necessary if column is to be reused.
- Collect all flow through samples for analysis if desired.

Procedure for the Determination of Dynamic Binding Capacity (DBC)

System Parameters

- Flow rate: 0.5-1 mL/min (or intended flow rate)
- Equilibrate: 10 CV loading buffer
- Sample load: Inject sufficient quantity of antibody-containing sample to exceed column capacity (if using an ÄKTAdesign system, use sample pump)
- Wash: 20 CV loading buffer
- Strip: 10 CV elution buffer
- Re-equilibration: 10 CV loading buffer
- Void volume (V_0): To determine V_0 , either perform a run in the bypass position (bypasses the column) or run the procedure using conditions which prevent protein binding. For example, use an elution buffer instead of a loading buffer for the equilibration and sample loading steps.

Calculation of AcroSep Column DBC

- Formula: $DBC = C \times (V_L - V_0)$

C = Concentration of load

V_L = Volume at 10% or 50% breakthrough

V_0 = From the void volume determination described above, V_0 is the total volume passing through the system from the time of injection (0% deflection of OD_{280}) until protein breakthrough (increase in OD_{280}).

Note: This protocol can be used for binding capacity determination for other proteins and/or different buffers.

Note: To convert the column DBC to resin DBC, divide the DBC value above by the column volume (1.04 mL resin).

Cleaning

General Cleaning

Column performance may decline over time due to incomplete removal of antibodies or contaminants. They are usually removed with the following procedure:

1. Wash column with 10 CV of loading buffer
2. Wash column with 5 CV of 1-2 M NaCl in buffer with a pH 4-8.5
3. Wash column with 10 CV of loading buffer

Cleaning Alkaline Soluble Materials

1. Wash column with 10 CV of loading buffer
2. Wash column with 1-5 CV of 0.1 M NaOH (less than 1 hr exposure to NaOH)
3. Wash column with loading buffer for 1 hr at 1-3 CV/hr

Sanitization

Between runs it may be necessary to remove pyrogens from the resin. The following procedure is recommended.

Standard Method

1. Wash column with 10 CV of loading buffer
2. Wash column with 1-3 CV of 0.1 M NaOH
3. Wash column with 10 CV of loading buffer

Alcohol/Acid Method

1. Wash column with 10 CV of loading buffer
2. Wash column with 1-3 CV of 1 M acetic acid/20% EtOH
3. Wash column with 10 CV of loading buffer

Storage Recommendations

- The column must be stored at 2-8 °C (36-46 °F) and cannot be frozen.
- Between runs, store the column at 2-8 °C (36-46 °F) in loading buffer.
- The storage buffer may also contain bacteriostatic agents such as 20% (v/v) ethanol and/or 1 M NaCl.

Adapter Recommendations

AcroSep pre-packed columns are made with a luer inlet and outlet for easy connection to syringes. The following table lists recommendations if adapters are needed to connect the columns to other types of tubing.

Connection To	Adapters (Upchurch Scientific*)
1/16" OD Teflon* and Tefzel* Tubing	1 kit (P-837) <i>Instructions provided with kit</i>
1/8" OD Teflon and Tefzel Tubing	1 kit (P-838) <i>Instructions provided with kit</i>
1/16" Stainless Steel Tubing	1 inlet fitting (P-658), 1 outlet fitting (P-655), 2 ferrules (P-259), 2 nuts (LT-115) <i>Instructions provided with fittings</i>
1/32" Stainless Steel Tubing	1 inlet fitting (P-658), 1 outlet fitting (P-655), 2 ferrules (P-248), 2 nuts (LT-115) <i>Instructions provided with fittings</i>

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