HiFliQ Protein A FPLC Columns

User Guide

Protein Ark's HiFliQ Protein A FPLC columns designed for simple, one-step and rapid antibody purification from serum, ascites and tissue culture supernatants. HiFliQ Protein A FPLC columns are supplied pre-packed and ready to use with high capacity Protein A Agarose FF providing broad source species and IgG subclass binding. Compatible with all common liquid chromatography instruments (including ÄKTA™ FPLC's), peristaltic pumps and syringes.

Table of contents:

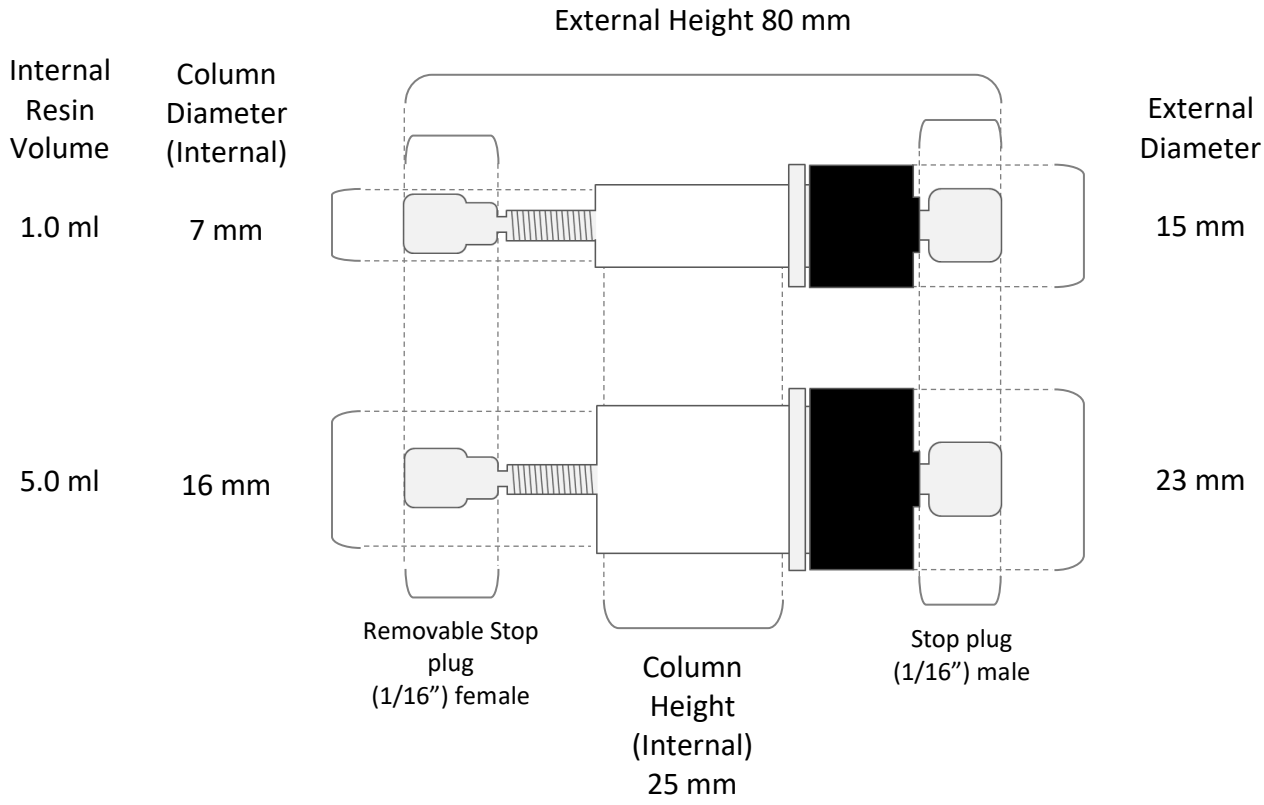
Features of the HiFliQ Protein A FPLC columns:

- Fast and reliable affinity purification.
- Pre-packed 1 ml and 5 ml columns for immunoglobulin affinity purification.
- Highly stable Agarose resin coupled with recombinant Protein A ligand provides high binding capacity with high buffer stability and minimal leaching.
- Broad source species (human, mouse, rabbit) and IgG subclass binding capability.
- Biocompatible polypropylene casing.
- Universal 10.32 (1/16'') UNF threads (Inlet Female/Outlet Male) compatible with all common chromatography instruments (including ÄKTA™ FPLC's).
- Compatible with low pressure pumps (requires a 1/16'' male connector) and syringes (requires a Luer Female – 1/16'' male connector).
- Connect in series for increased capacity.

Specification:

Item:	HiFliQ1-PA	HiFliQ5-PA
Column Volume:	1 ml	5 ml
Resin:	Protein A Agarose FF	Protein A Agarose FF
Base Matrix:	Agarose	Agarose
Coupled Ligand:	3.5 mg/ml resin Protein A	3.5 mg/ml resin Protein A
Leaching (measured by ELISA):	< 5ng Protein A /ml resin	< 5ng Protein A /ml resin
Typical Binding Capacity (hIgG):	30 mg	150 mg
Bead Size:	60-165 µm	60-165 µm
Recommended Flow Rate:	1 ml/min	1-5 ml/min
Max. operating pressure:	0.5 MPa (72 psi)	0.5 MPa (72 psi)
External Dimensions:	15 mm D. x 80 mm H.	23 mm D. x 80 mm H.
Column Dimensions (internal):	7 mm D. x 25 mm H.	16 mm D. x 25 mm H.
Column Construction:	Polypropylene	Polypropylene
Inlet Port:	10-32 (1/16") Female	10-32 (1/16") Female
Outlet Port:	10-32 (1/16") Male	10-32 (1/16") Male
Storage (2-8°C):	20% Ethanol	20% Ethanol

HiFliQ Protein A FPLC column schematic:



NOTE:	HiFliQ FPLC columns are supplied with a moulded removable (1/16") female stop plug attached to the (1/16") male outlet port. Remove prior to use, reverse and use to seal the column for storage.
NOTE:	HiFliQ columns cannot be opened or repacked.

Chemical compatibility:

HiFliQ Protein A FPLC columns shows high chemical resistance and are stable in all aqueous buffers commonly used for Protein A chromatograph and limited exposure to organic solvents (e.g. 70 % ethanol, 5.8 M acetic acid).

Buffer compatibility	
Standard Buffers:	Common aqueous buffers and salts
pH range:	2.5-10
Denaturants	
Urea:	8 M
Guanidinium hydrochloride:	6 M
Chaotropic agent	
Sodium isothiocyanate	2 M
Limited exposure	
Ethanol	70%
Acetic acid	5.8 M

Principles of Protein A and G chromatography:

All modes of chromatography can be used effectively for the separation of antibodies. Although ion-exchange chromatography can resolve different polyclonal antibodies and different subclasses, a degree of customization of the protocol is required. Affinity techniques include protein A or G, immobilized anti-antibodies and immobilized antigens. The simplicity of Protein A and Protein G affinity chromatography is extremely attractive and cost effective as it lends itself to the bind, wash and elute mode of operation using the appropriate buffer formulations. The use of proteins A and G is widespread, and has largely superseded the use of anti-antibodies.

Protein A is a cell wall protein from *Staphylococcus aureus* with a molecular weight between 35-50 kDa and shows high specificity for the Fc region of immunoglobulin molecules of many mammalian species. The quality of the resin (Protein A Agarose or equivalent) and stability of the immobilisation is important to avoid leakage of Protein A during the elution procedure.

Protein A affinity chromatography is a rapid one-step purification, which removes most non-IgG contaminants and can achieve purities close to homogeneity. It is particularly useful for purifications of tissue culture supernatant, where 10-100 fold concentrations can be achieved.

Protein A affinity resin:

Recombinant Protein A expressed in *E.coli* is covalently attached via the amino groups to Agarose at 3.5 mg Protein A per ml resin.

Applications of Protein A and G chromatography include:

Monoclonal antibody purification
Antibody concentration
Purification of polyclonal antibodies
Salt removal from antibodies
Removal of endotoxins from an antibody solution

General considerations for selecting optimal binding conditions for recombinant Protein A and Protein G resin:

Any sample, such as a crude biological extract, a cell culture supernatant, serum, ascites or an artificial standard can be used with the HiFliQ Protein A or Protein G FPLC columns. It is important that the sample is first filtered through a 0.45-1.2 µm syringe-end filter to remove particulates that could clog the resin flow channels. All samples should be filtered just prior to loading even if they have been filtered several days before the chromatographic run.

Aggregation/precipitation of proteins is common during storage and repeated freeze/thaw cycles in sera, ascites and tissue culture supernatants. Lipids, which can be found at high levels in serum or ascites should also be removed. Of equal importance is the ability to process the samples rapidly and, if the need arises, to be able to purify the target immunoglobulin at 4°C.

Optimal conditions for binding the target molecule to a resin are critical for successful separation of the protein. If the binding conditions are not optimal with respect to pH, salt concentration, presence or absence of metal ions etc, the interaction could be weak or non-existent. In many instances, the sample needs to be dialysed or diafiltered by ultrafiltration before it is applied to an affinity or ion exchange chromatographic support. In Protein A or G separations, the sample should simply be diluted 1:1 (v/v) in 1 x binding buffer.

Choosing between HiFliQ Protein A and G FPLC columns:

Immunoglobulin G from most species consists of several subclasses with different biological properties. Four subclasses of IgG have been identified in human (IgG1, IgG2, IgG3, and IgG4) and in mouse (IgG1, IgG2a, IgG2b and IgG3). For immunological studies, it is often necessary to isolate one particular subclass of IgG from the other subclasses.

Protein G binds to all major Ig classes except IgM and therefore has a wider reactivity profile than Protein A (see table 1 for details). However, the binding of Ig's to Protein G is often stronger, making elution and complete recovery of the immunoglobulin more difficult. Interestingly, due to the lower cost of Protein A compared to Protein G, researchers tend to experiment first with Protein A then Protein G. Protein A withstands harsher conditions used in cleaning and regeneration. The affinity of interaction of Protein A with mouse IgG subclasses varies. The most common subclass of mouse monoclonal antibodies is IgG1. Customization of the purification strategy may be required for the affinity separation as mouse IgG1 does not generally bind well to Protein A. However, as the affinity interaction is pH- and salt-dependent, under high salt regimes (2-3 M NaCl) and high pH (pH 8-9), the antibodies will bind to Protein A.

The needs of the researcher dictate that the speed of sample processing, the cost and the reproducibility are key criteria for selecting purification tools. By selecting immunoglobulin-binding proteins with the appropriate cross-reactivities coupled to careful design of the strategic protocol, objectives such as the selective recovery of therapeutic antibodies from complex mixtures is assured.

Table 1. Binding Affinities of Protein A and Protein G.

Isoform	Protein A	Protein G	Isoform	Protein A	Protein G
Human IgG1	✓✓✓✓	✓✓✓✓	Rabbit IgG	✓✓✓✓	✓✓✓
Human IgG2	✓✓✓✓	✓✓✓✓	Hamster IgG	✓	✓✓
Human IgG3	×	✓✓✓✓	Guinea Pig IgG	✓✓✓✓	✓✓
Human IgG4	✓✓✓✓	✓✓✓✓	Bovine IgG	✓✓	✓✓✓✓
Human IgA	✓✓	×	Sheep IgG	✓/×	✓✓
Human IgD	✓✓	×	Goat IgG	✓/×	✓✓
Human IgE	✓✓	×	Pig IgG	✓✓✓	✓✓✓
Human IgM	✓✓	×	Chicken IgG	×	✓
Mouse IgG1	✓	✓✓			
Mouse IgG2a	✓✓✓✓	✓✓✓✓			
Mouse IgG2b	✓✓✓	✓✓✓			
Mouse IgG3	✓✓	✓✓✓	**Fragments**	**Protein A**	**Protein G**
Mouse IgM	✓/×	×	Human Fab	✓	✓
Rat IgG1	×	✓	Human F(ab')2	✓	✓
Rat IgG2a	×	✓✓✓✓	Human scfv	✓	×
Rat IgG2b	×	✓✓	Hunam Fc	✓✓	✓✓
Rat IgG2c	✓	✓✓	Human κ	×	×
Rat IgM	✓/×	×	Human λ	×	×

Key code for relative affinity of Protein A & G for respective antibodies:

Strong Affinity	✓✓✓✓
Moderate affinity	✓✓✓
Weak affinity	✓✓
Slight affinity	✓
No Affinity	×

Monoclonal antibody purification:

This protocol describes the purification of IgG immunoglobulins from serum, ascites, or cell culture supernatants such as those derived from static cultures and bioreactors. It is recommended that the bind-wash-elute conditions be tested and optimised in order to achieve the optimum conditions for purification. All volumes are given in column bed volume (CV).

Example: Buffers for mouse IgG1 purification.

Binding buffer:
1.5 M Glycine/NaOH buffer, 3 M NaCl, pH 9.0
Elution buffer #1:
0.1 M Sodium citrate buffer, pH 5.5
Elution buffer #2:
0.2 M Glycine/HCl buffer, pH 2.5
Neutralization buffer:
1 M Tris/HCl buffer, pH 9.0

NOTE: The precise buffer conditions for your target protein may require scouting and optimizing relative to the species and subclass (see 'choosing the correct buffer conditions' for further details).
NOTE: Buffers can contain 0.1% sodium azide as a preservative for storage at room temperature.

Procedure

1. Prepare the sample by diluting the sample 1:1 (v/v) with 'Binding buffer'.

NOTE: Alternatively the sample can be dialysed, resuspend or buffer exchanged into 'Binding buffer' prior to loading.
NOTE: Delipidation may be required if antibody samples contain lipids and lipoproteins (e.g. samples from ascites fluid). See 'delipidation procedure' for details.

2. Connect the HiFliQ Protein A column to an FPLC/syringe/pump and equilibrate with 5-10 CV's of 'Binding buffer'.

NOTE: Remove and save the 'removable stop plug' at the column outlet prior to connecting the column.

3. Filter the sample through a 0.2 µm syringe filter directly before loading onto the column at the recommended flow rate.

4. After loading wash the column with 5-10 CV's 'Binding buffer' or until the measured absorbance (OD=280nm) reaches a stable baseline.

5. Elute the bound antibody with 2-5 CV's 'Elution buffer #1' (or 'Elution buffer #2') or until the measured absorbance (OD=280nm) reaches a stable baseline.

NOTE: If binding is too strong to be disrupted by 'Elution buffer #1' then repeat step 5 with 'Elution buffer #2'.

6. Immediately neutralize the pH of the eluted antibody with the 'Neutralization buffer.

NOTE: The antibody me be damaged if the pH is not neutralized immediately.
NOTE: For rapid neutralization, elute the bound antibody into fresh tubes containing the 'Neutralization buffer' at 100 µl per 1 ml elute or rapidly dialyse the eluted antibody in 100 x volume TBS overnight with a minimum of two dialysate changes.

7. Analyze the eluted antibody fractions by SDS-PAGE.

NOTE: Under reduced conditions the immumoglobulins will appear as two bands when run on an SDS-PAGE (IgG = 25 kDa and 50-55 kDa, IgM =25 kDa and 70-80 kDa).

NOTE: It is recommended that the column is re-used for identical antibodies to avoid cross-contamination.

Choosing the correct buffer conditions:

The interaction of immobilized Protein A with immunoglobulins (Ig's) is pH-dependent with an optimal binding capacity at pH 8-9. Salt concentration can significantly affect the binding of mouse Ig's to Protein A by reducing severe ionic interactions and enhancing hydrophobic interactions. Mouse IgG1, rat IgG1 and rat IgG2b bind well to immobilized Protein A when the salt concentration is higher than 1 M, but bind poorly at low salt concentrations. However, their binding capacities can be substantially lower than those antibodies that bind strongly to Protein A or G resin.

Typical binding buffers employed in Protein A affinity separations are:

1 -1.5 M Glycine/NaOH, 2-3 M NaCl, pH 9.0
1 M Sodium borate, 2 M NaCl, pH 9.0
0.1 M Sodium phosphate, 0.1 M NaCl, pH 7.4 (PBS)

Typical elution buffers employed in Protein A affinity separations are:

0.1 M Sodium citrate, pH 3.0-6.0
0.1-0.2 M Glycine/HCl, pH 2.5-3.0
0.1 M Sodium phosphate, pH 3.0-6.0

Typical neutralization buffer used for Protein A affinity separations is:

1M Tris/HCl, pH 9.0

Table 2. Affinity of Protein A for IgG subclasses.

Species	Subclass	Binding pH	Elution pH
Mouse	IgG1	8.5-9.0	6.0-7.0
Mouse	IgG2a	8.0-9.0	4.5-5.5
Mouse	IgG2b	8.0-9.0	3.5-4.5
Mouse	IgG3	8.0-9.0	4.0-7.5
Rat	IgG1	8.0-9.0	6.0-8.0
Rat	IgG2a	9.0	7.5-9.0
Rat	IgG2b	8.0-9.0	7.0-8.0
Rat	IgG2c	8.0-9.0	3.0-7.0
Human	IgG1	7.0-7.5	2.5-4.5
Human	IgG2	7.0-7.5	2.5-4.5
Human	IgG3	7.0-7.5	3.0-7.0
Human	IgG4	7.0-7.5	2.5-4.5
Rabbit	IgG	7.5	3.0-7.0
Guinea pig	IgG1	7.5-9.0	4.0-5.0
Guinea pig	IgG2	7.5-8.0	3.0-4.5

Delipidation:

Both Protein A and Protein G HiFliQ columns are affected by the presence of lipids and lipoproteins, especially in antibody samples derived from ascites fluid. For end users who have antibody solutions which they need to delipidate, the following protocol is a gentle and easy method for removing lipids and lipoproteins.

Procedure

1. Add 0.04 ml 10% dextran sulphate solution and 1 ml 1 M calcium chloride per ml sample.

2. Mix for 15 minutes.

3. Centrifuge at 10,000 x g for 10 minutes.

4. Discard the precipitate.

5. Buffer exchange the sample into TBS (Tris Buffered Saline) using dialysis, ultrafiltration or a desalting column.

NOTE: Do not buffer exchange into a phosphate-containing buffer such as PBS.

Desalting and concentrating the purified protein:

The purified IgG can be buffer exchanged or concentrated further using ultrafiltration concentrators for your downstream application.

Column washing and regeneration:

After each use insure that the column is carefully washed and regenerated correctly prior to another bind-wash-elute cycle. After regeneration, the column can be stored in storage buffer at 2-8°C until further use. All volumes are given in column bed volume (CV).

Example: buffers for washing and regeneration

Binding buffer:
1.5 M Glycine/NaOH buffer, 3 M NaCl, pH 9.0
Elution buffer #1:
0.1 M Sodium citrate buffer, pH 5.5
Storage buffer:
20% Ethanol (or 0.1% sodium azide)

Procedure: recommended after each run.

1. Wash the column with 10 CV's 'Elution buffer #1'.
2. Wash the column with 10 CV's 'Binding buffer'.
3. Wash the column with 5 CV's of 'storage buffer' for long term storage at 4°C.

NOTE: The column is now ready for re-use if required.

Performance data:

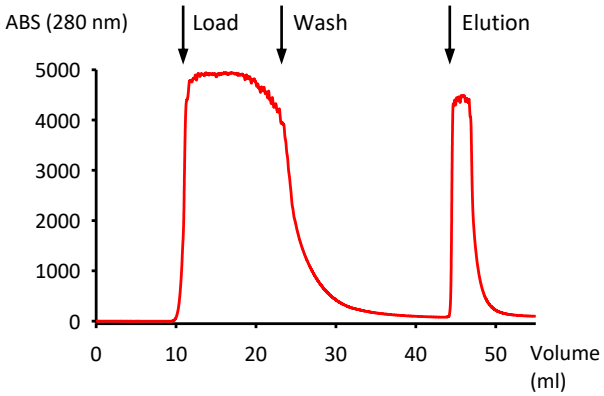
Purification of polyclonal rabbit IgG from serum.

Affinity purification of rabbit IgG's from rabbit serum using a 1 ml HiFliQ Protein A FPLC column on an FPLC. The eluted fraction contained >95% pure IgG antibody according to SDS-PAGE analysis (Figure 1B).

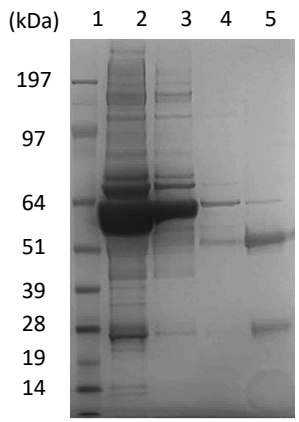
Sample:	6 ml rabbit serum
Column:	1 ml HiFliQ Protein A FPLC Column
Instrument:	FPLC
Flow rate:	1 ml/min
Binding buffer:	1 M Glycine, 2 M NaCl, pH 9.0
Elution Buffer:	100 mM Sodium Citrate, pH 2.75
Eluted IgG:	16.8 mg

Figure 1. Purification of rabbit IgG's from serum on a 1 ml HiFliQ Protein A FPLC column. (A) FPLC chromatogram (B) SDS-PAGE analysis.

A.



B.



SDS-PAGE using NuSep nUView 4-20% gradient, Quick Coomassie stained.

Lane 1: MW marker
Lane 2: Rabbit Serum
Lane 3: Flow-through
Lane 4: Wash pool
Lane 5: Eluted IgG

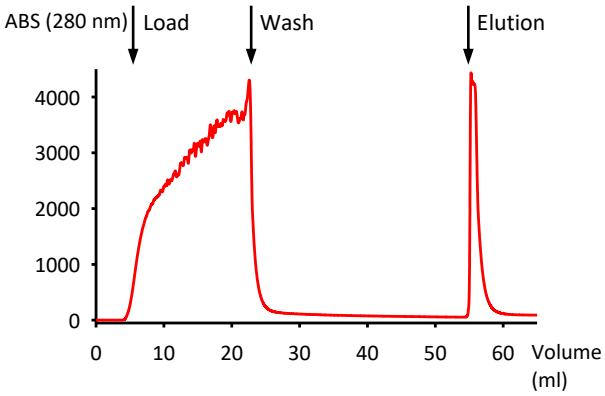
Purification of murine IgG_1 from cell culture supernatant.

Affinity purification of murine IgG_1 from hybridoma cell culture supernatant using a 1 ml HiFliQ Protein A FPLC column on an FPLC. The eluted fraction contained >95% pure IgG_1 antibody according to SDS-PAGE analysis (Figure 2B).

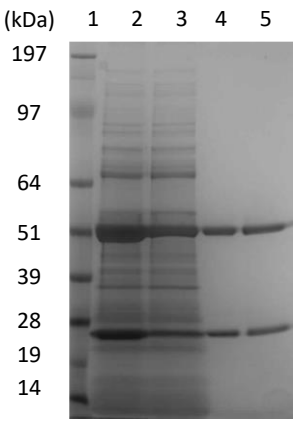
Sample:	50 ml (10x concentrated) cell culture supernatant
Column:	1 ml HiFliQ Protein A FPLC Column
Instrument:	FPLC
Flow rate:	1 ml/min
Binding buffer:	1 M Glycine, 2 M NaCl, pH 9.0
Elution Buffer:	100 mM Sodium Citrate, pH 2.75
Eluted IgG_1:	4.7 mg

Figure 2. Purification of murine IgG_1 from cell culture supernatant on a 1 ml HiFliQ Protein A FPLC column. (A) FPLC chromatogram (B) SDS-PAGE analysis.

A.



B.



SDS-PAGE using NuSep nUView 4-20% gradient, Quick Coomassie stained.

Lane 1: MW marker
Lane 2: Supernatant
Lane 3: Flow-through
Lane 4: Wash pool
Lane 5: Eluted IgG_1

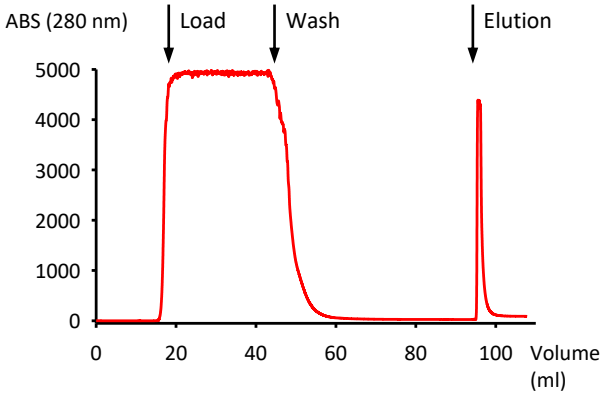
Purification of murine IgG_{2A} from cell culture supernatant.

Affinity purification of murine IgG_{2A} from hybridoma cell culture supernatant using a 1 ml HiFliQ Protein A FPLC column on an FPLC. The eluted fraction contained >95% pure IgG_{2A} antibody according to SDS-PAGE analysis (Figure 3B).

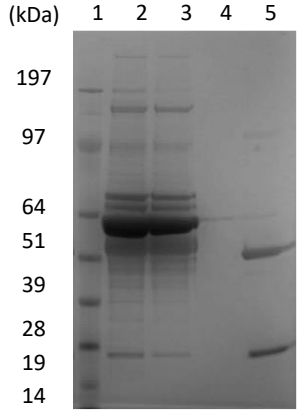
Sample:	20 ml (10x concentrated) cell culture supernatant
Column:	1 ml HiFliQ Protein A FPLC Column
Instrument:	FPLC
Flow rate:	1 ml/min
Binding buffer:	1 M Glycine, 2 M NaCl, pH 9.0
Elution Buffer:	100 mM Sodium Citrate, pH 2.75
Eluted IgG_{2A}:	5.0 mg

Figure 3. Purification of murine IgG_{2A} from cell culture supernatant on a 1 ml HiFliQ Protein A FPLC column. (A) FPLC chromatogram (B) SDS-PAGE analysis.

A.



B.



SDS-PAGE using NuSep nUView 4-20% gradient, Quick Coomassie stained.

Lane 1: MW marker
Lane 2: Supernatant
Lane 3: Flow-through
Lane 4: Wash pool
Lane 5: Eluted IgG_{2A}

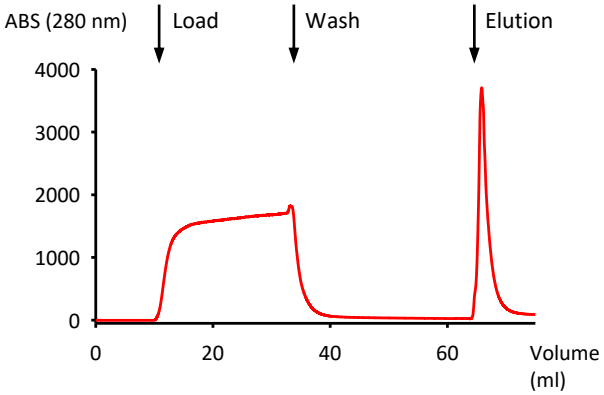
Purification of murine IgG_{2B} from cell culture supernatant.

Affinity purification of murine IgG_{2B} from hybridoma cell culture supernatant using a 1 ml HiFliQ Protein A FPLC column on an FPLC. The eluted fraction contained >95% pure IgG_{2B} antibody according to SDS-PAGE analysis (Figure 4B).

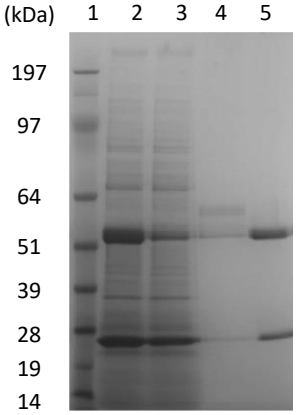
Sample:	50 ml (10x concentrated) cell culture supernatant
Column:	1 ml HiFliQ Protein A FPLC Column
Instrument:	FPLC
Flow rate:	1 ml/min
Binding buffer:	1 M Glycine, 2 M NaCl pH, 9.0
Elution Buffer:	100 mM Sodium Citrate, pH 2.75
Eluted IgG_{2B}:	4.8 mg

Figure 4. Purification of murine IgG_{2B} from cell culture supernatant on a 1 ml HiFliQ Protein A FPLC column. (A) FPLC chromatogram (B) SDS-PAGE analysis.

A.



B.



SDS-PAGE using NuSep nUView 4-20% gradient, Quick Coomassie stained.

Lane 1: MW marker
Lane 2: Supernatant
Lane 3: Flow-through
Lane 4: Wash pool
Lane 5: Eluted IgG_{2B}

Storage conditions:

Item:	HiFliQ1-PA	HiFliQ5-PA
Shipping:	20% Ethanol at 4°C	20% Ethanol at 4°C
Short-term storage (overnight):	Binding buffer at 4°C	Binding buffer at 4°C
Long-term storage:	20% Ethanol or 0.1% sodium azide	20% Ethanol or 0.1% sodium azide

NOTE:	Do not freeze or store the column at room temperature. Each column is stored in 20% Ethanol and is reusable for up to 2 years from the date of resin manufacture. Please see the label for the recorded expiry date.

Questions and answers:

1. *What is the shelf-life of the HiFliQ Protein A FPLC column?*
   The resin is guaranteed for 2 years after the date of manufacture provided they are stored at 2-8°C.

2. *Do I need to filter and degas the buffers prepared in my laboratory?*
   It is good laboratory practice to filter and degas all buffers.

3. *Do I need to pre-filter my sample before loading it on to the Protein A Agarose resin?*
   All samples should be filtered through a 0.45-1.2 µm syringe filter or centrifuged to remove particulates.

4. *What is the binding capacity of the HiFliQ Protein A FPLC columns?*
   We have been able to bind up to 30 mg/ml resin human IgG from serum for both the 1ml and 5 ml columns.

5. *What leaching levels can I expect from the HiFliQ Protein A FPLC columns?*
   We report leaching levels less than 5 ng Protein A/ml resin.

6. *How should I prepare my sample for loading onto the column?*
   Many chromatographic procedures demand that the sample is pre-conditioned prior to loading. Protein A affinity separations usually require the sample to be diluted 1:1(v/v) in 1 x binding buffer. We recommend that all samples are diluted 1:1 (v/v) in the binding buffer.

7. *Do I need to be careful with the type of chaotropic ion I use when eluting antibodies from affinity columns?*
   It is recommended to use the mildest chaotropic agents at the lowest possible concentration that will ensure rapid elution and high recovery of activity. Iodination reactions employing either chloramine T or IODO-GEN (1,3,4,6-

tetrachloro-3-6-diphenylglycouril) are particularly sensitive to inhibition by low concentrations of thiocyanate ions and, if antibodies are eluted from the HiFliQ Protein A FPLC column, using this chaotropic ion, it is essential that they are dialyzed thoroughly after elution to remove thiocyanate ions.

8. *How do I monitor purity of the isolated antibodies?*

Purity is best measured by gel electrophoresis. When analyzed by SDS-PAGE under non-reducing conditions, IgG antibodies should give a single protein band of about 160-170 kDa. On reduction with DTT or 2-mercaptoethanol, two or more bands will be seen corresponding to the individual heavy chains (50-55 kDa) or light chains (25-30 kDa). Other protein bands that are visible only on reduction may point to proteolytic action. This can often be prevented by careful use of protease inhibitors in culture supernatants before storage.

9. *How do I measure the final antibody concentration?*

You can use the Beer-Lambert law, A = ε.c.l, the concentration of IgG (mg/ml) in the sample can be measured by multiplying the absorbance at 280 nm by 0.72. If IgM or IgA are purified, multiply the absorbance at 280 nm by 0.84 or 0.94, respectively. These antibody concentrations are only estimates as other contaminating proteins can also contribute to the absorbance reading. However, they can provide a reliable and quantitative method for determining the concentrations of pure antibody solutions. Most researchers use a sandwich ELISA assay to accurately measure antibody concentrations within a range of 1 mg/ml to 20 mg/ml sample.

10. *What are the critical starting conditions for loading onto the HiFliQ Protein A and G FPLC columns?*

Sample pH and salt concentration are usually not critical. Typically the pH should be equal to or above 5.0 with a salt concentration equal to or over 0.1 M to prevent non-specific binding.

11. *Do I need to control the salt concentrations during Protein A and G chromatography?*

Use 0.1-0.5 M salt to reduce non-specific adsorption. When working with protein A, use high salt (2-3 M NaCl) with high pH to promote the binding of mouse IgG1.

12. *Is pH an important parameter to control during Protein A and G chromatography?*

The elution pH is the most critical variable. Protein G usually requires more acidic pH conditions to desorb the target immunoglobulins. For protein A, elution by pH steps (starting at pH 6) may fractionate different species (weaker binding bovine IgG from target antibodies) or subclasses. High pH (pH 8-9), in conjunction with high salt may promote binding of mouse IgG1 to protein A. The binding buffer pH should normally be higher than pH 6.0-7.0.

13. *Can I elute antibodies from the HiFliQ Protein A and G FPLC column using divalent cations?*

Concentrations of divalent cations (particularly Mg^{2+}) up to 1 M can sometimes replace acidic pH if there is concern about loss of activity of acid-labile immunoglobulins.

Troubleshooting assistance:

Bubbles or cracks appear in the resin bed

- The column has been stored at a cool temperature and then rapidly warmed up.
- The column should be warmed slowly to room temperature before use.

The sample does not flow easily through the column

- The column maybe clogged with particulates. Pre-filter the sample just before loading it on to the resin. Ascites must be delipidated before use (see earlier procedure).
- If the column is not stored at 2-8°C, or they have been used more than once and stored in the absence of a bacteriostat, microbial growth may restrict flow through the resin.

No elution of the target protein is observed from the column

- The pH of the elution buffer may be incorrect. It is advisable to prepare new solutions.
- The elution conditions are too mild to desorb the target protein.

The recovery of target protein is low

- The binding of antibodies to Protein A or G is attributed in part to hydrophobic forces. Use chaotropic salts to reduce the strength of all hydrophobic interactions.

Poor resolution of the target protein

- The sample volume or concentration may be too large for the capacity of the column. In this case, reduce the sample load or sample volume.
- The sample may also need to be filtered carefully.

The target protein elutes at an unexpected position

- There may be an ionic interaction between the protein and resin. You should maintain the ionic strength above 50 mM.
- There may be hydrophobic interactions between the sample and resin. In this instance, reduce the salt concentration and add suitable detergents or organic solvents.
- The column may be dirty.

The elution profile cannot be reproduced

- The nature of the sample may have altered and so it may be important to prepare a fresh sample.
- The sample load may be different from the original sample load. It is advisable to keep all these parameters constant.
- Proteins or lipids may have precipitated in the resin bed. Use elution conditions which stabilize the sample.
- The buffer pH and ionic strength is incorrect and new buffers will need to be prepared.

Glossary:

affinity chromatography - chromatographic separation based on a specific interaction between an immobilized ligand and a binding site on a macromolecule.

antibody - an immunoglobulin produced by the immune system of vertebrates in response to exposure to a foreign substance.

antigen - a molecule which can bind specifically to an antibody.

antiserum - the serum fraction from an animal that has been immunized or exposed to an immunogen and contains antibodies to a particular antigen.

ascites - a liquid tumour formed by injection of a hybridoma cell line into the peritoneal cavity. It is a common source of monoclonal antibodies from mice.

column bed volume (CV) - the total volume occupied by the chromatographic packed bed. It is also referred to as the column volume or CV.

cell culture supernatant - the fluid made during cell culture (either roller bottle, suspension or perfusion) containing tissue media components and the secreted target.

chaotropic agent - a molecule which interferes with hydro-phobic interactions by disrupting the ordered structure of water molecules. Examples include urea and guanidine.

hybridoma - a hybrid cell line produced by fusing antibody producing cells with myeloma cells to generate immortal reproducing cells that produce specific monoclonal antibodies indefinitely in cell culture.

immobilized - bound to a surface, usually through covalent linkages.

immunoglobulin (Ig) - comprising 5 distinct classes in most higher animals. Classes called IgG (the most common), IgM, IgA, lgD and IgE. They differ from each other in size, charge, amino acid composition and carbohydrate content.

ion exchange chromatography - chromatographic separation based on different charge properties of macromolecules.

isoelectric point - the pH at which the protein has no net charge.

monoclonal antibody - an antibody derived from a single clone of immune cells. They are usually formed from a hybridoma cell line.

polyclonal antibodies - antibodies produced to the same immunogen by different cell types. Antibodies from antiserum are almost always polyclonal.

protein A/protein G - cell wall proteins of certain pathogenic bacteria which specifically bind to the Fc region of immunoglobulins.

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Ordering information:

Product	Volume	Order Code
1 ml HiFliQ Protein A FPLC column, 1 x 1 ml	1 x 1ml	HiFliQ1-PA-1
1 ml HiFliQ Protein A FPLC columns, 5 x 1 ml	5 x 1ml	HiFliQ1-PA-5
5 ml HiFliQ Protein A FPLC column, 1 x 5 ml	1 x 5 ml	HiFliQ5-PA-1
5 ml HiFliQ Protein A FPLC columns, 5 x 5 ml	5 x 5 ml	HiFliQ5-PA-5

Accessories	Quantity	Order Code
Proteus Protein A Buffer Pack (250 ml 1x Binding buffer (A) pH 9.0, 125 ml 1 x Elution Buffer (B1) pH 5.5, 125 ml 1x Elution Buffer (B2) pH 2.5 30 ml Neutralization Buffer (C) pH 9.0)	1	PC-BAP

Technical support:

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