Protein L Agarose High Flow and Pre-Packed Column

Protein L has strong affinity to the variable region of antibody kappa light chains (V_κ). Although it binds to the Fab portion of immunoglobulins, Protein L does not interfere with antigen-binding sites. Therefore, Protein L potentially can be used in immunoprecipitation (IP) procedures.

As a good complementary choice for Protein A and Protein G, Protein L has been used for purification of IgM, IgE, IgD, IgA and IgY, in addition to IgG. It is especially advantageous in purification of antibody fragments such as Fabs, single-chain variable fragments (scFv), and domain antibodies (Dabs). Some characteristics of Protein I:

- Binding to Vκ, which complements Protein A and G (binding to Fc)
- broad binding specificity: it binds to poly and monoclonal antibodies and antibody fragments from a wide range of different species and subclasses
- No bovine serum interference: it does not bind to bovine immunoglobulin, can be used to purify recombinant antibodies from media containing bovine serum.
- Single point attachment coupling chemistry that gives better ligand accessibility for higher binding capacity

Description

Protein L Agarose High Flow Resin is manufactured by immobilizing recombinant Protein L ligand to highly cross-linked agarose matrix through stable bond formed by epoxy coupling chemistry. Protein L ligand is derived from E Coli fermentation and binds tightly to the variable region of kappa light chain of a wide range of immunoglobulins. Protein L can bind to kappa types I, III, and IV of human IgG and kappa type I of mouse IgG. Besides IgG, Protein L has been used for efficient purification of IgM, IgE, IgD, IgA and IgY containing kappa light chains. The resin especially shows great advantage in purification of antibody fragments such as Fabs, single-chain variable fragments (scFv), and domain antibodies (Dabs). Please refer to the Table 1 for specifications of Protein L Agarose High Flow.

Instructions

1. Column packing

Protein L Agarose High Flow is supplied as a suspension in 20% ethanol. Decant the ethanol solution and exchange it with water or other packing buffer before use.

Table 1. Characteristics of Protein L Agarose High Flow

Composition	Highly cross-linked Agarose
Average particle size	90 micron
Form	Slurry in 20% Ethanol 1ml/5ml prepacked column (available, please inquire)
Ligand	recombinant Protein L
Coupling chemistry	Epoxy
Dynamic binding capacity	> 5-10 mg human IgG /ml media
Recommended flow rate	100-300cm/hr
Recommended column height	5-20cm
Maximum Pressure Drop	0.3MPa
Chemical stability	Stable in all commonly used aqueous buffers
pH working range	2-10
pH CIP range (short term)	2-11
CIP stability	8M Urea, 10mM NaOH + 1M NaCl, 0.1M acetic acid
Temperature stability	2-40 °C (shipping at RT. Store at 2-8 °C)
Storage	20% Ethanol
Shelf life	5 years

Table 2. Characteristics of Protein L Prepacked Column

Composition	Highly cross-linked Agarose
Average particle size	90 micron
Form	1ml/5ml prepacked column
Ligand	rProtein L
Coupling chemistry	Epoxy
Dynamic binding capacity1	5-10mg human IgG /1ml prepacked column; > 25-50mg human IgG /5ml prepacked column
Recommended flow rate	0.2-1 ml/min for 1ml prepacked column 1-5ml/min for 5ml prepacked column
Column dimension	0.7x2.5 cm for 1ml prepacked column 1.6x2.5 cm for 5ml prepacked column
Maximum Pressure Drop	0.3Mpa
Chemical stability	Stable in all aqueous buffers commonly used in protein L chromatography
pH working range	2-10
pH CIP range (short term)	2-11
CIP stability	8M Urea, 10mM NaOH + 1M NaCl, 0.1M acetic acid
Temperature stability2	2-40 °C (shipping at RT. Store at 2-8 °C)
Storage	20% Ethanol
Shelf life	5 years

1. Equilibrate all material to the purification temperature. Assemble the column (and packing device, if necessary).

2. Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air is trapped under the column net. Close the column outlet leaving the net covered with packing buffer.
3. Resuspend the medium stored in its container by shaking (avoid stirring the sedimented medium). Mix the packing buffer with the medium to form a 50% to 70% slurry (sedimented bed volume/total slurry volume = 0.5 to 0.7).
4. Pour the homogeneous slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will help to minimize the introduction of air bubbles.
5. If using a packing device, immediately fill the remainder of the column and packing device with packing buffer. Mount the adapter or lid of the packing device and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
6. Open the bottom outlet of the column and turn on the pump to run at the desired flow rate. Ideally, Protein L resin is packed at a constant pressure of approximately 1 bar (0.1 MPa). If the packing equipment does not include a pressure gauge, use a packing flow rate of approximately 400 cm/h (10 cm bedheight, 25°C, water as packing buffer). If the recommended pressure or flow rate cannot be obtained, use maximum flow the pump can deliver.
7. When the bed height has stabilized, mark the compressed bed height and close the bottom outlet and stop the pump.
8. If using a packing device, disconnect the packing device and mount the adapter to the column.
9. With the adapter inlet open, push the adapter down, approximately 2 mm below previous compressed bed height, and the packing buffer will flush the adapter inlet. Close the adapter inlet.
10. The column is now ready for use.

Note: Please do not exceed 75% of the packing flow rate in subsequent chromatographic purification procedures.

2. Purification

Binding Affinity

Binding characteristics of Protein L ligand are summarized in Table 3. Please use it as a general guide for media selection.

Recommended purification parameters

Generally, antibodies and fragments bind Protein L Agarose High Flow resin at neutral pH and physiological ionic strength, and are eluted at low pH. The recommended buffers for purification listed below can be used as starting conditions for your experiments:

Table 3 Binding characteristics of Protein L ligand

Species	Subclass	rProtein L	Species	Subclass	rProtein L
Human	Total IgG	+++	Mouse	Total IgG	+++
	IgG1	++++		IgG1	+++
	IgG2	++++		IgG2a	+++
	IgG3	+++		IgG2b	+++
	IgG4	++++		IgG3	+++
	IgA	+++		IgM	+++
	IgA1	+++	Rat	Total IgG	+++
	IgA2	+++		IgG1	+++
	IgD	+++		IgG2a	+++
	IgE	+++		IgG2b	+++
	IgM	+++		IgG2c	+++
Cow	Total IgG	-		IgG3	?
	IgG1	-	Hamster		+++
	IgG2	-	Rabbit	Total IgG	+
Horse	Total IgG	?	Chicken	IgY	+
Goat	IgG	-	Cat	Total IgG	?
	IgG1	-	Dog		?
	IgG2	-	Pig		+++
Sheep	Total IgG	-	Guinea-pig	IgG1	?
	IgG1	-		IgG2	?
	IgG2	-	Koala		?
Monkey(rhesus)	IgG	?	Llama		?

++,Strong binding; +, medium binding; -, weak or no binding

Recommended buffers:

Binding buffer:

- 20mM Sodium phosphate, 150mM NaCl, pH7.2
- 20mM Tris, 100mM NaCl, pH7-8
- Phosphate buffered saline (PBS), pH 7.4 (0.01M phosphate buffer, 0.0027M KCl, 0.14M NaCl)

Elution buffer:

- 100mM Glycine, pH 2.5-3.0

Neutralization buffer:

- 1M Tris pH 8-9

Purification procedures:

1. Pack the column as described in “Column Packing” section. The recommended column height is within 5-20cm.
2. Equilibrate the column at recommended flow rate with 5-10 column volumes of binding buffer to get a stable baseline.
3. Calculate appropriate sample amount for loading. In principle, dynamic capacity is affected by many parameters, such as antibody type, residence time, sample concentration, binding buffer and so on. Therefore, the maximum loading volume can be obtained by frontal analysis for individual sample under specific binding conditions. Generally, the dynamic binding capacity is around 15-25mg Fab per ml medium for 4-8min residence time.
4. Please note that there might be considerable deviations in binding capacity for different immunoglobulins derived from the same species, even if they are of the same subclass.
5. Apply clarified sample of antibody onto column. Samples need to be clarified by 0.45-micron filter to remove any particles and colloids before application. It is recommended to dilute samples of high protein concentration, such as anti-serum, with equal volume of binding buffer to reduce sample viscosity.
6. Wash column with 5 column volumes of binding buffer until UV level drop to baseline. Though not necessary for most of the cases, optional intermediate washing step with salts or detergents may help to remove impurities.
7. Elute the column with 10 column volumes of elution buffer. The most commonly used elution pH is 3.0; however, pH 2.5-3.0 is required for efficient elution of strong binding antibodies with high recovery. Non-ionic detergents, arginine and urea have been reported to improve antibody stability and avoid aggregation during elution.
8. Neutralize the elution peak immediately with 1M Tris buffer of pH 8.0-9.0.
9. Re-equilibrate the column with 5-10 column volumes of neutral binding buffer.

3. Clean in place (CIP)

Clean in place (CIP) is important for removing tightly bound, precipitated or denatured proteins, DNA and lipids, so as to maintain performance and capacity of the column. Protein L Agarose High Flow Resin allows the use of low concentration of NaOH as CIP agent. A recommended CIP procedure:

1. Wash the column with 3 to 5 column volumes of binding buffer.
2. Backflush with 1 to 2 column volumes of CIP buffer with contact time of 10 minutes. Three commonly used CIP buffers are listed below:
   - 0.1M glycine pH 2.5
   - 8M Urea
   - 10mM NaOH
3. Wash immediately with 5-10 column volumes of binding buffer at pH 7-8 to remove CIP reagents.

CIP is usually performed immediately after the elution. Before applying the alkaline NaOH CIP

solution, we recommend equilibrating the column with a solution of neutral pH in order to avoid the direct contact between low-pH elution buffer and high pH NaOH solution on the column. Mixing acid and alkaline solutions might cause a rise in temperature in the column. Cleaning reagents concentration, contact time and frequency are typically the main parameters to vary during the optimization of the CIP. The nature of the feed material will ultimately determine the final CIP. However, the general recommendation is to clean the column at least every 5 cycles during normal use. Depending on the nature of the contaminants, different protocols may have to be combined, for example 10mM NaOH every cycle, and 8M Urea or 0.1M glycine pH2.5 every 10-20 cycles. 8M Urea can remove precipitated proteins to restore performance. 1M NaCl can be introduced into CIP regents for stabilizing the ligand under alkaline conditions.

4. Sanitization

Sanitization reduces microbial contamination of the chromatography column. Protein L Agarose High Flow Resin allows use of 0.1M acetate acid in 20% ethanol as sanitizing agent. A recommended sanitization procedure:

1. Wash the column with 3 column volumes of binding buffer.
2. Wash with 0.1 M acetic acid in 20% ethanol for sanitization. Contact time of one hour is recommended.
3. Wash immediately with at least 5 column volumes of sterile and filtered binding buffer at pH 7-8.

5. Trouble shooting

High column backpressure during purification

- Disconnect the column with system and make sure no tubings or connectors in the system caused the high system pressure; always use tubings and connectors of right inner diameters
- Remove flow restrictor from systems if possible
- Calibrate the pressure sensor in your systems
- Make sure all buffers and samples are filtered through 0.22 or 0.45 micron disc membrane for clarification. For small volume sample, 10000g@10-20min centrifugation is an alternative solution
- Lower flow rate when use buffers of high viscosity or working at cold temperature, especially during sample loading
- Replace top screen net of column adapter in case of clogging
- Lower the column bed height to 20 cm or less, too high beds will cause high pressure
- Perform a thorough CIP procedure to restore the initial backpressure if column bed clogs. Unpack the column and wash media batch wise
- Increase the CIP frequency and optimize the CIP regent formulations
- Avoid freeze the medium or column during storage

Poor binding or low capacity

- Check the binding affinity of your antibodies of interest to the ligand
- Make sure the pH values of binding buffer and sample are pH 7-8
- Check if there exist some interference substances in binding buffer or samples, such as high concentration of chaotropic substances
- Lower flow rate to give a residence time of 4-8min for sample loading
- Check the history of the medium about how it has been cleaned and stored.

Inefficient elution

- Check the pH value and composition for elution buffer
- Try elution buffer of lower pH, for example pH 2.3
- Use some chaotropic substances of low concentration in elution buffer
- Introduce some solvents to decrease the polarity
- Try other affinity media or other technologies

Low purity

- Reduce the sample holding time, lower purification temperature and always use protease inhibitors in samples and buffers to avoid degradation
- Try to use as mild as possible elution conditions to avoid antibodies aggregation, and be sure to neutralize peak collected immediately after elution
- Introduce an intermediate washing step before elution to remove any
- non-specific binding impurities, and some commonly used substances in washing buffer includes 1M NaCl, 0.5M Tetramethylammonium Chloride or detergents.
- Use pH linear 5-10 column volumes gradient (for example, phosphate and citrate to form pH 7.3 to 2.3 gradient) instead of stepwise elution and pool fractions of high purity
- Alternative chromatography techniques need to be combined with affinity chromatography for higher purity with a multistep purification strategy, such as size exclusion and ion-exchange, etc.

Storage

Store unused media in its container at 2 to 8°C. Ensure that the container is closed and fully tightened. Equilibrate packed columns with 5-10 column volumes of 20% ethanol to prevent microbial growth. A thoroughly CIP procedure is recommended before long-term storage. Please **never freeze the media**.