FabAffinity® KBP High Flow Beads

FabAffinity KBP High Flow Beads, with KBP (human Kappa chain binding protein) coupled to high flow Agarose matrix, is a new choice for purification of human antibodies and antibody fragments containing the constant domain of the Kappa light chain.

This in-house developed proprietary ligand, derived from HEK293 cell, can bind to the constant domain of the kappa light chain (i.e. fragments lacking the constant region of the light chain will not bind), therefore capable of binding target molecules containing the constant region of the light chain, for example, Fab, IgG, IgA and IgM.

KBP ligand has very good chemical compatibility to common chromatography buffers and cleaning solutions, such as 10mM NaOH, 6M Urea, 0.1M Acetate acid and 30% IPA, which is favorable for repeated CIP cycles without significant loss of capacity.

Features:

- High kappa chain binding affinity validated by SPR with Kd in the nmol range
- Efficient capture of human antibodies and antibody fragments containing the constant region of the Kappa light chain, even from complex sources at very low concentrations.
- Good chemical stability and easy cleaning with long life time
- Single point attachment coupling chemistry gives better ligand accessibility for higher binding capacity

1, Description

Antibody fragments are gaining more and more attention as potential biopharmaceuticals because of advantages over monoclonal antibodies (MAbs), such as improved pharmacokinetics for tissue penetration and binding to targets which are inaccessible to conventional antigen-binding sites.

KBP ligand (human Kappa Chain Binding Protein), an in-house developed proprietary affinity ligand, is expressed from HEK293 cell and interacts with the constant region of human kappa light chain of a wide range of immunoglobulins, therefore capable of binding target molecules containing the constant region of the light chain, for example, Fab, IgG, IgA and IgM.

FabAffinity KBP High Flow Beads, as part of Acrobiosystems' FabAffinity Family, is manufactured by immobilizing recombinant KBP ligand to highly cross-linked agarose matrix through stable bond formed by epoxy coupling chemistry.

FabAffinity KBP High Flow Beads has been used for both excellent chromatography purification of human antibodies and fragments in one step, but also immunoprecipitation to purify and detect proteins or protein complexes successfully.

Please refer to the following Table 1 for specifications of FabAffinity KBP High Flow Beads in details.

Table 1. Characteristics of FabAffinity KBP High Flow Beads

Composition	Highly cross-linked Agarose
Average particle size	90 micron
Form	Slurry in 20% Ethanol 1ml/5ml prepacked column Spin column kit (5 cycles)
Ligand	rKBP derived from HEK293
Coupling chemistry	Epoxy
Dynamic binding capacity[1]	10mg Fab (Mr.50kDa) /ml media
Recommended flow rate	100-300cm/hr
Recommended column height	5-20cm
Maximum Pressure Drop	0.3MPa
Chemical stability	Stable in all aqueous buffers commonly used in KBP affinity chromatography
pH working range	3-10
pH CIP range (short term)	2-11
CIP stability	6M Urea, 5mM NaOH, 0.1M acetic acid, 30% IPA
Temperature stability[2]	2-40 °C
Storage	20% Ethanol
Shelf life	5 years

1, Determined at 10% breakthrough at 4min residence time

2, Delivered at room temperature, and recommended long-term storage at 2-8 °C

Table 2. Characteristics of FabAffinity KBP Prepacked Column

Composition	Highly cross-linked Agarose
Average particle size	90 micron
Form	1ml/5ml prepacked column;
Ligand	rKBP derived from HEK293
Coupling chemistry	Epoxy
Dynamic binding capacity[1]	10mg Fab /1ml prepacked column; 50mg Fab /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 KBP chromatography
pH working range	3-10
pH CIP range (short term)	2-11
CIP stability	6M Urea, 5mM NaOH, 0.1M acetic acid, 30% IPA
Temperature stability[2]	2-40 °C
Storage	20% Ethanol
Shelf life	5 years

1, Determined at 10% breakthrough at 4min residence time

2, Delivered at room temperature, and recommended long-term storage at 2-8 °C

2, Instructions for chromatography purification

2.1 Column packing

FabAffinity KBP High Flow Beads is supplied as a suspension in 20% ethanol. Decant the 20% ethanol solution and exchange it with water or other packing buffer required before use. Then follow the procedures below:

1. Equilibrate all material to the temperature at which the purification will be performed. 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 has been 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, FabAffinity KBP 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 bed height, 25° C, water as packing buffer).

If the recommended pressure or flow rate can not be obtained, use the 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 to use for purification.

Note:

Do not exceed 75% of the packing flow rate in subsequent chromatographic purification procedures.

2.2 Purification

2.2.1 Binding Affinity

KBP interacts with the constant region of human kappa light chain of a wide range of immunoglobulins, therefore capable of binding target molecules containing the constant region of the light chain (CL-kappa), for example, Fab, IgG, IgA and IgM. Please refer to the following Figure 1,2 and Table 3 for separation media selection of antibody fragments purification. For more detail information about FabAffinity family resins, please visit our website.

Figure 1 Structure of antibodies and fragments

Figure 2 Affinity constant of KBP to kappa chain in nmol range (SPR)

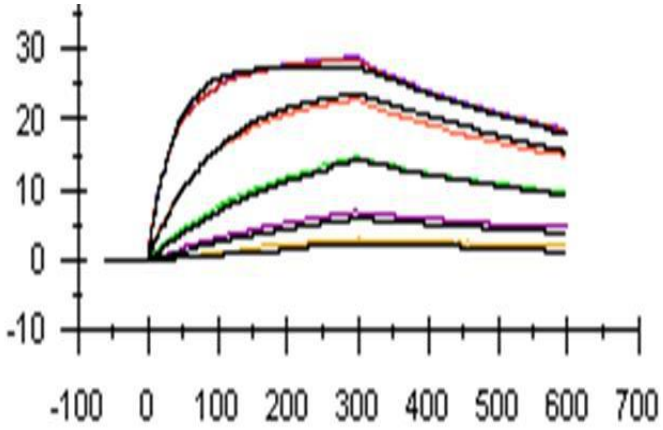

Table 3 Guide of affinity media selection for antibody fragments purification

Antibody fragment	Fab		ScFv	Domain Antibody	
Chain Subtype	Kappa light chain	Lambda light chain	Kappa light chain	Kappa light chain	VH3 heavy chain
Recommended products	Protein L or **KBP**	LBP	Protein L	Protein L	Protein A
Binding site	VL-kappa or **CL-kappa**	CL-Lambda	VL-kappa	VL-kappa	VH3

The dynamic binding capacity is a function of the sample residence time. It is necessary to use appropriate linear flow rate during sample application to ensure that residence time is in 4 to 8 min range at optimal column height of 5 to 20 cm. The residence time is equal to the packed bed height (cm) divided by the linear flow rate (cm/h) applied during sample loading.

2.2.2 Recommended purification parameters

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

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

- Neutralize 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 related to lots of 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 /ml medium for 4-8min residence time.

Note:

Please note that there might be considerable deviations in binding capacity for different antibodies and fragments.

4. Apply clarified sample onto column. Samples need to be clarified by 0.45micron 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.

5. 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 to some extent.

6. Elute the column with 10 column volumes elution buffer. The most commonly used elution buffer is pH3.0; however, pH 2.5-3.0 is required for efficient elution of some kind of very strong binding moleculars with high recovery. Arginine and urea have been reported to improve target molecular stability and avoid aggregation during elution.

7. Neutralize the elution peak immediately with 1M Tris buffer of pH 8.0-9.0.

8. Re-equilibrate the column with 5-10 column volumes of neutral binding buffer.

2.3 Clean in place (CIP)

Clean in place (CIP) is the important procedures for removing very tightly bound, precipitated or denatured proteins, DNA and lipids, so as to maintain performance and capacity of the column.

FabAffinity KBP High Flow Beads allows using the following CIP procedures:

CIP procedures:

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, and three commonly used CIP buffers are listed below for selection:
   - 0.1M acetate acid
   - 6M Urea
   - 5mM 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. 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 0.1M acetate acid every cycle, and 6M Urea or 5mM NaOH every 5-10 cycles. 6M Urea can remove the precipitated proteins to restore the performance for resin. 1M NaCl can be introduced into CIP regents for stabilizing the ligand under alkaline conditions.

2.4 Sanitization

Sanitization reduces microbial contamination of the chromatography column to a minimum. FabAffinity KBP High Flow Beads allows the use of 0.1M acetate acid in 20% ethanol as sanitizing agent for sanitization.

Sanitization procedures:

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.

2.5 Trouble shooting

1. 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 be 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 back pressure 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

2. Poor binding or low capacity

- Check the binding affinity of your molecules 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.

3. 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, such as 10-30% IPA
- Try other affinity media or other technologies

4. 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 target molecules 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.

3, Instructions for immunoprecipitation (IP)

3.1 Additional material required

- 1.5-2 ml micro centrifuge tube
- IP Buffer: 25mM Tris, 150mM NaCl, pH 7.2
- Antigen Sample: Antigen-containing lysate or sample prepared in IP Buffer or other buffer that is compatible with both the desired antibody binding interaction and the binding of antibody or antibody fragment to KBP
- Elution Buffer: 0.1 M glycine, pH 2-3
- Electrophoresis Loading Buffer: Lane marker reducing sample buffer (4X)
- Neutralization Buffer: 1 M Tris-HCl pH8-9

3.2 Immunoprecipitation procedures

- Combine 50-1,000 μl of the Antigen Sample and optimized amount of antibody or antibody fragment in a microcentrifuge tube. Incubate overnight at 4° C.
- Add 100 μl of KBP Agarose slurry to the antigen-antibody complex. Incubate with gentle mixing for 4 hours at 4° C.

Note: Always keep samples on ice.

- Add 0.5 ml of IP Buffer, centrifuge for 2 minutes at 3,000×g and discard supernatant. Repeat this step for several times.
- Add 50 μl of Elution Buffer and incubate for 5 minutes to elute the immune complex. Centrifuge for 2 minutes at 3,000×g and collect the supernatant. Repeat this step and combine the two supernatant fractions.
- Adjust eluate to physiological pH by adding Neutralization Buffer. The IP products can be used directly for SDS-PAGE, or buffer exchanged by dialysis or desalting column for the specific downstream application.

4, Storage

Store unused media in its container at a temperature of 2 to 8° C for long term storage. 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. After storage, equilibrate with binding buffer and ready for use.
Never freeze the media in case of generation of fine particles to increase the back pressure.

5, Ordering Information

Cat No	Product Description	Size
FK-0422-C1	FabAffinity KBP Prepacked Column 1ml	1ml
FK-0422-C5	FabAffinity KBP Prepacked Column 5ml	5ml
FK-0422-01	FabAffinity KBP High Flow Beads	5ml

Other related product:

Cat No	Molecule	Product Description	Host	Size
RPA-S3149	Protein A	Recombinant Protein A Protein	E Coli	2mg/10mg
RPG-S3140	Protein G	Recombinant Protein G Protein	E Coli	2mg/10mg/5g
RPL-P3141	Protein L	Recombinant Protein L Protein	E Coli	2mg/10mg/5g
RPA-S8149	Biotinylated Protein A	Biotinylated Protein A	E Coli	2mg
RPG-S8140	Biotinylated Protein G	Biotinylated Protein G	E Coli	2mg
RPL-P814R	Biotinylated Protein L	Biotinylated Protein L	E Coli	500 μg/2mg
BZ-E0226	Benzonase	DNA and RNA Nuclease (Ultra Pure, Protease Free)	E Coli	10kU/50kU /500kU
HC-E0226	HRV-3C	HRV-3C Protease Cleavage Enzyme, With GST Tag	E.Coli	1kU/2.5kU/50kU

Ordering online:

Please visit our website at www.acrobiosystems.com and find the product by Cat. No. or search the product name, then make the payment with credit card or Paypal.

Ordering offline:

Please send us your inquiries or POs by E-mail or Fax with the following information, and we will get back to you within 24hr:

- Name & full contact details including telephone, fax, and email address.
- Name of your Institution /Company
- Shipping Address
- Billing Address
- Purchase Order Number or Complete Credit Card Information
- Catalog Number of Each Product
- Size/ Quantity of Each Product

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6, Technical support

If you have any technical questions regarding quality or application of the products, please contact us via E-mail, phone or fax. Our experienced application scientists will try best to answer your questions or solve your problems as soon as possible.

E-mail: Techsupport@acrobiosystems.com