Super Protein A Agarose High Flow Resin

Cat. No. COP27 **Lot. No.** (See product label)

SPECIFICATION

PRODUCTINFORMATION	Product Overview: Super Protein A Agarose High Flow Resin belongs to the resins family for capture of monoclonal antibodies (MAbs), and is available with different pack sizes for easy Mabs purification, process development and scale up. Immunoprecipitation studies with Protein A Agarose High Flow are also commonly used to purify proteins or protein complexes.Description: Protein A Agarose High Flow Resin is a Protein A affinity medium for cost effective and efficient purification of monoclonal and polyclonal antibodies at any scale. With the novel NaOH tolerant modified rProtein A , i.e. Protein A as ligand, Protein A Agarose High Flow Resin allows the use of rigorous and cost-effective CIP and sanitization protocols based on 0.1 to 0.5 M NaOH. Moreover, compared with common rProtein A, the Protein A ligand is more protease stable leading to much lower ligand leakage and longer lifetime.Features: NaOH-tolerant Protein A ligand withstands rigorous CIP and sanitization procedures with 0.1 to 0.5 M NaOH, which contributes to significant time and cost saving for easy operation and much longer lifetime. Modified ligand structure design gives enhanced protease resistance resulting in lower ligand leakage so as to simplify the consequent Mab polish procedure. Generic mild elution conditions help to retain conformation and function of monoclonal antibodies during purification cycle. Single point attachment coupling chemistry gives better ligand accessibility for higher binding capacity.Specifications:Note: Determined at 10% breakthrough at 4min residence time. Delivered at room temperature, and recommended long-term storage at 2-8 °C.
INSTRUCTIONS	1. Column packing: Protein A Agarose High Flow is supplied as a suspension in

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FORPURIFICATION

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, Protein A 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 antibody purification.Note: Do not exceed 75% of the packing flow rate in subsequent chromatographic purification procedures.2. Purification2.1 Binding AffinityAs a modified version of traditional Protein A ligand,

innovative Protein A ligand has similar IgG binding specificity and affinity, and offers high selectivity and resolution for efficient capture of antibodies.Binding characteristics of Protein A ligand are summarized in Table 2, which can be used as general guide for affinity separation media selection of antibody purification. Even for a single specific subclass, there might be substantial diversity in binding characteristics. Lots of protocols have been developed to achieve enhanced binding for antibodies purification with high capacity and recovery, such as increasing pH value and salt concentration in binding buffers to reduce electrostatic repulsion and promote hydrophobic interactions between ligand and target antibody. 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 Purification parametersGenerally, IgG from most species and Fc-fusion protein bind Protein A Agarose High Flow resin at neutral pH and physiological ionic strength, and are eluted at low pH.Table 3 Recommended Protein A resin binding and elution conditions for human and mouse IgGsThe recommended buffers for purification listed below can be used as good starting conditions for your experiments: (1). 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). (2). Elution buffer: 100mM Glycine, pH 3.0; 100mM Acetate acid, pH 3.0; 20mM Citric acid, pH 3.0. (3). Neutralize buffer: 1M Tris pH 8-9.Note: For some weakly binding IgGs, such as mouse IgG1, please use the following buffers for purification as starting conditions to promote binding to rProtein A resin: (1). Binding buffer: 50mM Tris, 1M glycine, 0.5M NaCl, pH 9.0. (2). Elution buffer: 100mM sodium acetate, pH 4.5.2.3 Purification procedures1. 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

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	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 higher than 15mg Fab /ml medium for 4-8min residence time.4. Apply clarified sample of antibody 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 antibodies with high recovery. Arginine and urea have been reported to improve antibody stability and avoid aggregation.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.
PURIFICATION CASES	Sample: Clarified HEK293 SFM cell culture containing human IgG1 mAb, 321mg/L titerBinding buffer: PBS pH 7.3Elution buffer: 100mM Glycine pH 3.0Column: Protein A Agarose High Flow Resin packed into a 2.6x 10 (cm, i.d. x Height) 50ml columnLoad sample volume: 4.2L, totally 1348mg human IgG1 mAb (Elisa)Flow rate: 26ml/min (300cm/hr) for loading, 13ml/min(150cm/hr) for elutionUV280nm detection.Peak neutralized immediately with 1/20(v/v) 1M Tris pH8.0 after elutionPurity assay by 13% reduced SDS-PAGE4.2L SFM HEK293 cell culture 321 mg/L titer (transient expression with Proprietary HEKMax Technology) was loaded onto Protein A Agarose High Flow Resin for human

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	IgG1 mAb capture. And finally 1340mg mAb (BCA Assay) with >95% purity was obtained successfully.
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.Protein A Agarose High Flow Resin, a innovative NaOH-tolerant protein A medium, allows the use of NaOH as CIP agent, which is widely accepted for CIP due to low cost and efficient cleaning ability for protein, saponify fats and endotoxin. Recommended CIP procedures are as below:ClP procedures:(1). Wash the column with 3 to 5 column volumes of binding buffer.(2). Backflush with 1 to 2 column volumes of 6 M Urea or 0.1-0.5 mM NaOH with contact time of 10 minutes, and three commonly used CIP buffers are listed below for selection: 6 M Gua-HCl; 8 M Urea; 0.1-0.5 M NaOH.(3). Wash immediately with 5-10 column volumes of binding buffer at pH 7-8 to remove ClP 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 jn temperature in the column.NaOH 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 0.1 M NaOH every cycle and 0.5 M NaOH every 10 cycles. 1M NaCl or 5% Sucrose can be introduced into CIP regents for stabilizing the ligand under alkaline conditions. Denaturants such as 6M Gua-HCl and 8M Urea can remove the precipitated proteins, and non-ionic detergents and solvents such as 70% Ethanol can remove hydrophobic substances.

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SANITIZATION	Sanitization reduces microbial contamination of the chromatography column to a minimum. Protein A Agarose High Flow Resin allows the use of NaOH as sanitizing agent for effectively inactivating viruses, bacteria, yeasts, and endotoxins.Sanitization procedures:1. Wash the column with 3 column volumes of binding buffer.2. Wash with contact time of at least 10 minutes for 0.5 M NaOH, or 30 minutes for 0.1 M NaOH or contact time of one hour for 0.1 M acetic acid in 20% ethanol.3. Wash immediately with at least 5 column volumes of sterile and filtered binding buffer at pH 7-8.Note: Higher concentrations of NaOH and/or longer contact time inactivate microorganisms more effectively. However, these conditions might also lead to a dramatic decrease in the dynamic binding capacity. The conditions for sanitization should therefore be balanced between maximizing microbial killing and minimizing loss of capacity.
TROUBLE SHOOTING	High column backpressure during purification1. 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 diameters2. Remove flow restrictor from systems if possible3. Calibrate the pressure sensor in your systems4. 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 solution5. Lower flow rate when use buffers of high viscosity or working at cold temperature, especially during sample loading6. Replace top screen net of column adapter in case of clogging7. Lower the column bed height to 20 cm or less, too high beds will cause high pressure8. Perform a thorough CIP procedure to restore the initial back pressure if column bed clogs. Unpack the column and wash media batch wise9. Increase the CIP frequency and optimize the CIP regent formulations10. Avoid freeze the medium or column during storage.Poor binding or low capacity1. Check the binding affinity of your antibodies of interest to the ligand.2. Make sure the pH values of binding buffer and

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	sample are pH 7-8.3. Adjust the binding buffer and sample condition to promote binding: increase salt concentration to 0.5-1M NaCl, increase pH to 8.5-9.0, etc.4. Check if there exist some interference substances in binding buffer or samples, such as high concentration of chaotropic substances.5. Lower flow rate to give a residence time of 4-8min for sample loading.6. Check the history of the medium about how it has been cleaned and stored.Inefficient elution1. Check the pH value and composition for elution buffer.2. Try elution buffer of lower pH, for example pH 2.3.3. Use some chaotropic substances of low concentration in elution buffer.4. Introduce some solvents to decrease the polarity.5. Try other affinity media or other technologies.Low purity1. Reduce the sample holding time, lower purification temperature and always use protease inhibitors in samples and buffers to avoid degradation.2. Try to use as mild as possible elution conditions to avoid antibodies aggregation, and be sure to neutralize peak collected immediately after elution.3. 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.4. Use pH linear 5-10 column volumes gradient (for example, phosphate and citrate to form pH 7.3 to 3.0 gradient) instead of stepwise elution and pool fractions of high purity5. 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 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

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	media in case of generation of fine particles to increase the back pressure.

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