Protein A Agarose High Flow Resin

Cat. No. COP26 **Lot. No.** (See product label)

SPECIFICATION

PRODUCTINFORMATION	Product Overview: RProtein A Agarose High Flow Resin is a classic protein A affinity medium for fast and efficient purification of monoclonal and polyclonal antibodies at any scale. The high specificity of rProtein A ligand to Fc domain of IgGs gives a high purification factor in a single chromatography step. Superior coupling chemistry ensures both high capacity and low leakage. RProtein A Agarose High Flow resin has also been used in immunoprecipitation (IP) procedures successfully.Description: RProtein A Agarose High Flow Resin is manufactured by immobilizing recombinant rProtein A ligand to highly cross-linked agarose matrix through stable bond formed by epoxy coupling chemistry. RProtein A ligand is derived from E. Coli fermentation and interacts with antibodies through two distinct binding events: the "classical" primary binding site on the Fc region of human IgG1, IgG2, and IgG4, and the "alternate" secondary binding site on the Fab region of human IgG, IgM, IgA, and IgE that contain heavy chains of the VH3 subfamily. RProtein A Agarose High Flow has been used for both excellent chromatography purification of poly and monoclonal antibodies from several species of mammals in one step, but also immunoprecipitation to purify and detect proteins or protein complexes indirectly through antibodies against the protein or protein complex of interest successfully.Features: Classic rProtein A resin suitable for different applications, including purification of poly or monoclonal antibodies of different species and subclasses and isolation of immune complex. 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

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	storage at 2-8 °C.
INSTRUCTIONS FORPURIFICATION	1. Column packing: RProtein A Agarose High Flow is supplied as a suspension in 20% ethanol. Decant the 20% ethanol solution and replace 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 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 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

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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 AffinityClassic rProtein A ligand has versatile IgG binding specificity and affinity, and offers high selectivity and resolution for efficient capture of antibodies. Binding characteristics of rProtein 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. Especially for those IgGs of lower binding affinity to rProtein A, such as mouse IgG1, 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 20cm. 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 rProtein A 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 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

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	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 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 CHO SFM cell culture containing human IgG1 mAb, 45mg/Ltiter.Binding buffer: 50mM Tris 100mM NaCl pH7.6Elution buffer: 100mM Glycine pH 3.5Column: RProtein A Agarose High Flow Resin packed into a 1.6x 5(cm, i.d. x Height) 10ml columnLoad sample volume: 2.2L, totally 99mg human IgG1 mAb (Elisa)Flow rate: 5ml/min (150cm/hr) for loading and elutionUV280nm detection.Peak neutralized immediately with 1/20(v/v) 1M Tris pH8.0 after elution.Purity assay by 13% reduced gradient SDS-PAGE.2.2L SFM CHO cell

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	culture with 45mg/L titer (transient expression with Proprietary CHOMax Technology) was loaded onto rProtein A Agrose High Flow Resin for human IgG1 mAb capture. And finally 98mg 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. Recommended CIP procedures for rProtein A Agrose High Flow Resin 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 8 M Urea or 10 mM NaOH with contact time of 10 minutes.(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. CIP 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 Gua-HCl every 5 cycles.Denaturants such as 8M Urea can remove the precipitated proteins, and non-ionic detergents and solvents such as 70% Ethanol can remove hydrophobic substances.
SANITIZATION	Sanitization reduces microbial contamination of the chromatography column to a minimum. RProtein A Agarose High Flow Resin 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.

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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 diameters.2. Remove flow restrictor from systems if possible.3. Calibrate the pressure sensor in your systems.4. 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.5. Lower flow rate when use buffers of high viscosity or working at cold temperature, especially during sample loading.6. Replace top screen net of column adapter in case of clogging.7. Lower the column bed height to 20 cm or less, too high beds will cause high pressure.8. Perform a thorough CIP procedure to restore the initial back pressure if column bed clogs. Unpack the column and wash media batch wise.9. Increase the CIP frequency and optimize the CIP regent formulations.10. 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 sample are pH 7-8.3. Adjust the binding buffer and sample conditions to promote binding: increase salt concentrations 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

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	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 purity.5. Alternative chromatography techniques need to be combined with affinity chromatography for higher purity with a multistep purification strategy, such as size exclusion and ionexchange, 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 media in case of generation of fine particles to increase the back pressure.

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