Genetel Laboratories, LLC ***Leader in Proteomics Abs and Abs Arrays***
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GAPAR-Hu Anti-Human Plasma Total Protein Column Kit

Description

GAPAR-Hu is an anti-normal human plasma total proteins column for affinity depletion (removal) of global abundant proteins from the plasma samples to facilitate proteomic study for identifying new protein markers. The advantage of GAPAR (Global Abundant Proteins Affinity Removal) over MARS (Multiple Affinity Removal System) is that GAPAR removes most, if not all, abundant proteins and retains the low abundant and non-antigenic proteins while MARS is designed for removing only a few known (up to 20) abundant proteins. Depending on the samples, about 90-95% protein mass could be removed by GAPAR-Hu. GAPAR-Hu is highly recommended for identification of new plasma protein markers associated with diseases. The column can be re-used for multiple times after regeneration. The kit is highly suitable for downstream proteomic analysis methods such as LC-MS/MS. It is easy to use and the whole process will take less than one hour. The column can be regenerated and re-used many times. We have tested the column and found similar depletion efficiencies after 10 regenerations.

Product Components and Storage Condition

2 GAPAR-Hu spin columns; column size: 0.5ml resins in PBS with 0.05% Na Azide; store at 4^{o}C up to one year; binding capacity is about 2.5mg/ml resin.
3 2mL collecting tubes for spin columns, store at room temperature
1 Microcon Ultracel YM-3 for concentrating proteins, store at room temperature

Material/Equipment needed

Human plasma or serum samples (5ul sample per kit)
PBS with or without 0.05% Na Azide or other suitable buffer that will not interfere antigen-antibody binding
Elution Buffer: 0.1M Citrate/Na Citrate, pH2.5-3 (15.4g Citric acid, 5.9g Na_3 Citrate in 1L DI Water, adjust pH to 2.5)
Microcentrifuge

GAPAR-Hu Protocol

You will need to pass your plasma sample through the two columns to obtain efficient depletion of abundant proteins. Therefore, you should mark your column (such as 1 and 2) in order to remember which one has your sample on. You may do this in cool room or 4^{o}C condition if desired.

1. Prepare plasma sample: add 5ul plasma sample into 95ul PBS; filter through a .45μm filter if desired.

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2. Remove the top cap then the bottom cap from the columns to drain the column. Make sure that there are no resins inside the top cap, and if so, use PBS to rinse the resins into the columns. Put the bottom cap back after the buffer surface reaches the top of resins.
3. Apply the 100ul prepared plasma sample onto one column, and 100ul PBS onto the second column. Put the top cap back.
4. Mix for 20 minutes. You may need to tap the side of column so that resins and samples are completely mix, and then inverting the columns.
5. After the 20 minutes mixing, loose the top cap with a half turn then remove the bottom cap and put the columns into the 2ml collecting tubes immediately.
6. Spin the columns in a microcentrifuge for 10 seconds.
7. Immediately transfer the flow through plasma sample from the first column into the second column and mix well by tapping on the side of column. You can add about 300ul buffer into the first column and mix, which will be used for balancing the centrifugation later.
8. After 20 minutes of mixing, loose the top cap with a half turn then remove the bottom cap and put the columns into the new 2ml collecting tubes immediately. Make sure to mark the tube so that you know which one will contain your sample.
9. Spin the columns in a microcentrifuge for 10 seconds.
10. You will obtain about 300ul-400ul column unbound, low abundant proteins with a concentration of about 50-100ug/ml.

Concentrating low abundant proteins

If desired, you may concentrate the proteins to your require volume or amount using the provided Microcon Ultracel YM-3. Transfer your sample into the Microcon, cap the tube and balance with another 1.5ml tubes for centrifugation. Spin for about 20-60 minutes until it reaches your desired amount.

Column Re-generation

Immediately after spinning, add 300-400ul Elution Buffer into the column and mix well by tapping on the side of column. Spin for 10 seconds and monitor the OD280. Repeat this at least 5 times until OD280 is less than 0.050. After completing the elution, wash the column 6 times with 300-400ul PBS with 0.05% Na Azide by the same method. At the last step, reconstitute the column in 300-400ul PBS with 0.05% Na Azide and store the column in 4^{o}C.

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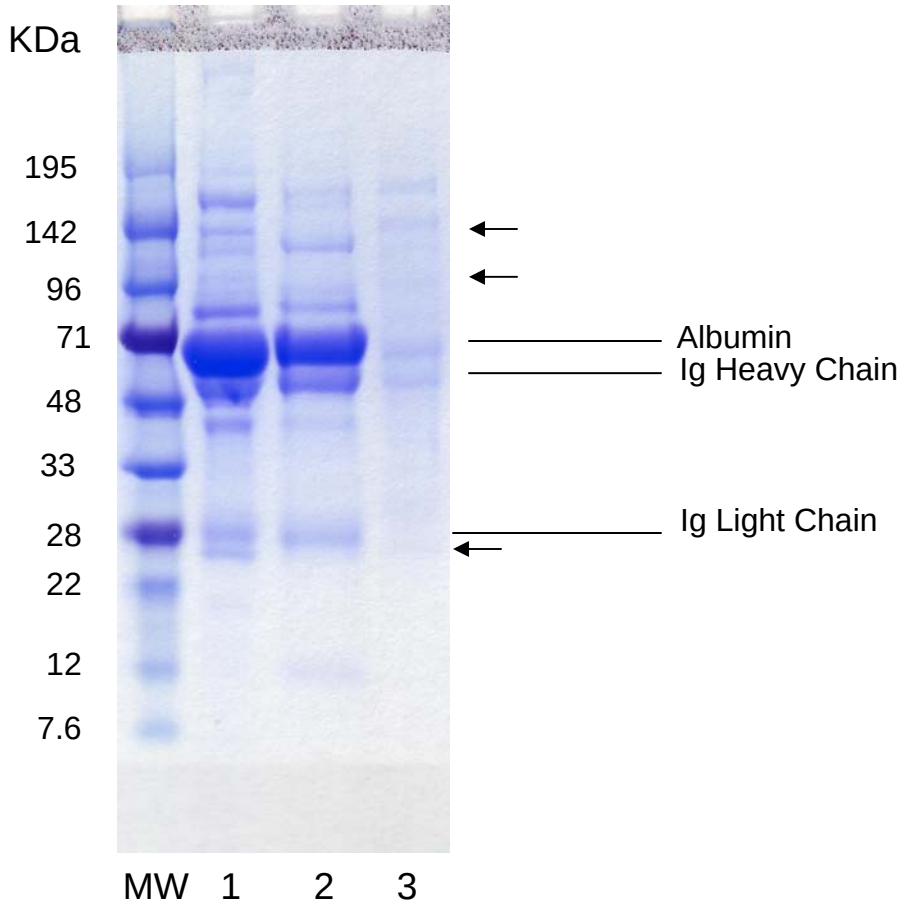

SDS-PAGE Gel Analysis of human plasma sample (lane 1), the column-bound proteins (lane 2) and the column flow through proteins (lane 3). Five micro-liter of plasma was diluted with PBS to 100ul and loaded on the columns for affinity depletion of abundant proteins. The total volume of both column-bound proteins and flow-through proteins were adjusted to 100ul, the same volume as the diluted original plasma sample. Each lane was loaded with 15ul of respective sample (lane 1: diluted original plasma; lane 2: eluted column-bound proteins; lane 3: the column flow-through proteins). Arrows indicate the proteins (weak bands) that did not bind to the GAPAR-Hu column, which could be low abundant proteins. About 95% of total protein mass was depleted by the GAPAR-Hu kit.