Ion Exchange PhyTip® Columns

PhyTip® Columns

This specification sheet provides details on PhyTip® Columns containing Ion Exchange Chromatography Resin. PhyTip® columns are unique capture, purification and enrichment tools from Biotage designed for micro-volume protein sample preparation. PhyTip® columns are available for a variety of liquid handling platforms and contain specific affinity resins for application specific requirements. Ion exchange PhyTip® columns are PhyTip® columns packed with ion exchange resins for purification of protein samples.

Samples for purification and enrichment must be clear and free from particulate matter. It is highly recommended to centrifuge samples and use the clear supernatant only, prior to use with PhyTip® columns.

PhyTip® columns are available in two formats, 200+ with a recommended maximum sample volume of 200 µL and 1000+ with a recommended maximum volume of 1000 µL. For each of the PhyTip® column formats there are several different resin volumes available. Each PhyTip® column has been designed for maximum efficiency of capture and elution of the specific protein(s) of interest when using the specified protocol. See below.

Shipping and Storage

Each pack of PhyTip® columns has been manufactured and qualified to the highest standards and shipped in retainer boxes that maintain the integrity of the specific affinity resin within each PhyTip® column. This product is shipped at ambient temperatures, but on receipt should be stored in a standard laboratory refrigerator between 4 and 8 °C.

- Do NOT freeze or store frozen.
- When not in use, keep the lid of the box closed and sealed, store in the refrigerator.
- Do not allow affinity resin to dry out by extended storage in a dry environment.

PhyTip® columns with ion exchange resin columns are shipped in a storage buffer containing glycerol. Interstitial storage buffer in the column may drip out during shipment or storage to form an opaque resin bed. The resin will still be hydrated in this state unless the bed has visibly shrunk. The resin has dried when the bed has visibly shrunk and only then is it recommended not to use the PhyTip® columns. If this occurs, please contact your regional sales representative.

Important Product Information

The packed column of the PhyTip® can cause pressure to build up within the tip. This internal pressure must be compensated for at each aspirate and dispense step. This is especially important when working with small volumes.

- 1000+ format
  - If you need to process a volume ‹ 250 µL, add 230 µL to that volume.
  - Example: A 200 µL volume should be programmed as 430 µL (200 + 230).
- 200+ format
  - If you need to process a volume ‹ 75 µL, add 40 µL.
  - Example: A 10 µL volume should be programmed as 50 µL (10 + 40).

Prevent aspirating or dispensing air in the PhyTip® column by only mixing 95% of the volume within the well.

» Example: Aspirate and dispense 950 µL of a 1000 µL sample

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Ion Exchange PhyTip® columns have been optimized for use with specific Biotage instrument flow rates/volumes as shown below. This information was collected using the MEA 2 Personal Purification System.

Biotage recommends processing samples with the following buffers (not supplied):

Equilibration Buffer:
Equilibrate the columns to a suitable pH. Select a pH higher than the target protein's pI when using Anion Exchange Resin or a pH lower than the pI of the protein when using a Cation Exchange Resin.

Wash Buffer:
Wash in a pH buffer equivalent to the Equilibration Buffer.

Step Elution Gradient:
Prepare a step gradient of the appropriate number of fractions. Each fraction is based upon the Equilibration Buffer with an increasing concentration of NaCl.

1000+ PhyTip® columns with Ion Exchange Resin

Process a 1000 µL sample using the conditions shown below.

Equilibration:
1000 µL Equilibration Buffer processed by passing through the resin bed for two cycles at a flow rate of 500 µL per minute with a 20 second pause at the end of each aspirate and each dispense step.

Capture:
1000 µL sample captured by passing through the resin bed for four cycles at a flow rate of 500 µL per minute with a 20 second pause at the end of each aspirate and each dispense step.

Wash:
1000 µL Wash Buffer, passed over the resin bed for two cycles at a flow rate 500 µL per minute with a 20 second pause at the end of each aspirate and each dispense step.

Elute:
Process the columns with a stepwise elution in Elution Buffer of increasing salt concentration. Each fraction of 2-3 times the resin bed volume is processed with 4 cycles at a flow rate of 500 µL per minute with a 20 second pause at the end of each aspirate and each dispense step. Repeat for the total number of fractions.

200+ PhyTip® columns with Ion Exchange Resin

Process a 200 µL sample using the conditions shown below.

Equilibration:
200 µL Equilibration Buffer processed by passing through the resin bed for two cycles at a flow rate of 250 µL per minute with a 20 second pause at the end of each aspirate and each dispense step.

Capture:
200 µL sample captured by passing through the resin bed for four cycles at a flow rate of 250 µL per minute with a 20 second pause at the end of each aspirate and each dispense step.

Wash:
200 µL Wash Buffer, passed over the resin bed for two cycles at a flow rate 250 µL per minute with a 20 second pause at the end of each aspirate and each dispense step.

Elute:
Process the columns with a stepwise elution in Elution Buffer of increasing salt concentration. Each fraction of 2-3 times the resin bed volume is processed with 4 cycles at a flow rate of 250 µL per minute with a 20 second pause at the end of each aspirate and each dispense step. Repeat for the total number of fractions.

Example of Lysozyme purification with Strong Cation Exchange PhyTip® Columns

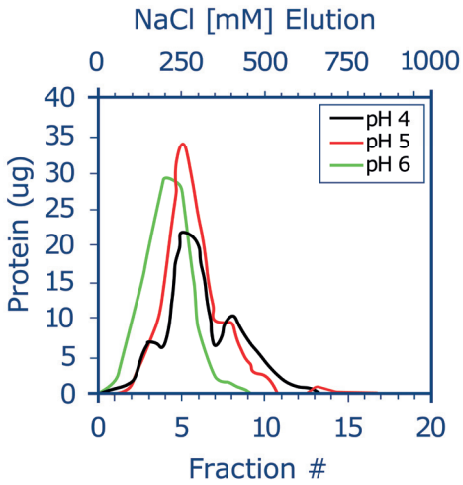

Figure 1. Lysozyme was spiked into 200 µL of PBS buffer of various pHs. 200+ PhyTip® columns containing 5 µL Strong Cation Exchange Resin was used to process the samples. Following Equilibration, Capture and Wash, the columns performed 20 step elutions of 15 µL consisting of Equilibration Buffer ranging from 50 to 1000 mM NaCl (each step was increased by 50 mM NaCl). Fractions were measured for absorbance at 280 nm using the NanoDrop spectrophotometer.

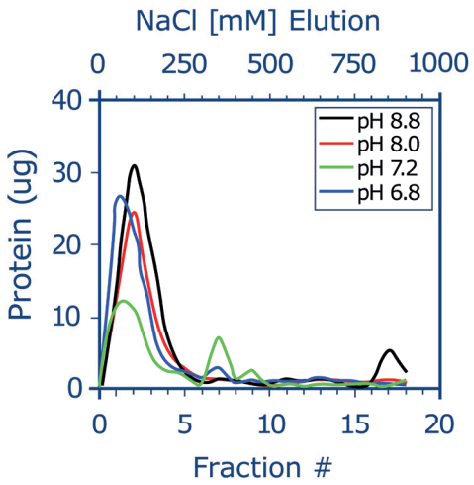

Figure 2. BSA was spiked into 200 µL of PBS buffer of various pHs. 200+ PhyTip® columns containing 5 µL Weak Anion Exchange Resin wasused to process the samples. Following Equilibration, Capture and Wash, the columns performed 20 step elutions of 15 µL consisting of Equilibration Buffer ranging from 50 to 1000 mM NaCl (each step was increased by 50mM NaCl). Fractions were measured for absorbance at 280nm using the NanoDrop spectrophotometer.

US Patent Nos: 7,482,169; 7,488,603; 7,722,820; 7,837,871; 7,875,462; 7,943,393; 8,057,668; 8,148,168

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