Streptavidin PhyTip® Columns

PhyTip® Columns

This specification sheet provides details on Streptavidin PhyTip® columns.

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. Streptavidin PhyTip® columns are packed with Streptavidin affinity resin for purification of biotin-labeled proteins.

Biotage recommends the use of biotin-labeled bovine albumin (Sigma, A8549) as a standard to verify the use of PhyTip® columns with all applications.

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.

Streptavidin PhyTip® 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

Calibration tips can be requested free of charge from Biotage.

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Table 1. PhyTip® Column Binding Capacity

Resin bed volume	Recommended protein binding capacity for highest recovery by dual flow chromatography
5 µL	3 µg
10 µL	7 µg
20 µL	13 µg
40 µL	27 µg
80 µL	53 µg
160 µL	107 µg
320 µL	213 µg

Streptavidin PhyTip® columns have been optimized for use with general molecular biology laboratory reagents and specific Biotage instrument flow rates/volumes as shown below. This information was collected using the MEA 2 Personal Purification System.

Purifying Biotinylated Molecules

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

Capture Buffer:
Phosphate Buffer solution pH 7.4, for those situations where additional buffer needs to be added to supplement the volume of the sample and to ensure correct pH for capture.

Wash Buffer I:
Phosphate Buffer solution pH 7.4.

Wash Buffer II:
Phosphate Buffer solution pH 7.4.

Elution Buffer:
A high salt and acidic buffer such as 8M guanidinium HCl pH 1.5.

Neutralization buffer
Tris Buffer solution pH **9.0**

Performing Immunoprecipitation or Pull-Down Assay

Biotage recommends the following buffers (not supplied):

Capture Buffer:
Phosphate Buffer solution pH 7.4, for those situations where additional buffer needs to be added to supplement the volume of the sample and to ensure correct pH for capture.

Wash Buffer I:
Phosphate buffer solution pH 7.4.

Wash Buffer II
Phosphate buffer solution pH 7.4 .

Elution Buffer:
A high salt buffer or an acidic buffer such as Phosphate buffer solution pH 2.5.

Neutralization Buffer
Tris buffer solution pH **9.0**

1000+ Streptavidin PhyTip® Columns

For a 500 µL sample with 10 µg biotin-albumin spiked into PBS buffer and processed using the conditions shown below, greater than 80% of the original biotin-albumin mass is immobilized.

A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip® column at 1–2 mm above the bottom of the well.

Equilibrate:
1000 µL of Equilibration buffer, passed over the resin bed for two cycles at a flow rate 500 µL/min.

Capture:
500 µL sample captured by passing through the resin bed for four cycles at a flow rate of 500 µL/min.

Wash:
1000 µL of PBS, passed over the resin bed for two cycles at a flow rate 500 µL/min.

Affinity Pull-Down:
500 µL sample captured by passing through the resin bed for four to eight cycles at a flow rate of 500 µL per minute. (For lower affinity pull-downs we recommend twelve to sixteen cycles).

Elute:
Elute the protein into solution with a volume of three times the resin bed volume of elution buffer, passed over the resin bed for four cycles at a flow rate of 500µL/min.

Neutralize:
Neutralize with 1M Tris pH 9.0 using a volume equal to ¼ of the elution volume. Mix.

200+ Streptavidin PhyTip® Columns
For a 200 µL sample with 10 µg biotin-albumin spiked into PBS buffer and processed using the conditions shown below, greater than 80% of the original biotin-albumin mass is immobilized.

Equilibrate:
200 µL of Equilibration buffer, passed over the resin bed for two cycles at a flow rate 250 µL/min.

Capture:
200 µL sample captured by passing through the resin bed for four cycles at a flow rate of 500 µL per minute.

Wash:
200 µL of PBS, passed over the resin bed for two cycles at a flow rate 500 µL/min.

Affinity Pull-Down:
200 µL sample captured by passing through the resin bed for four to eight cycles at a flow rate of 500 µL per minute.

Elute:
Elute the protein into solution with a volume of three times the resin bed volume of elution buffer, passed over the resin bed for four cycles at a flow rate of 500 µL/min.

Neutralize:
Neutralize with 1M Tris pH 9.0 using a volume equal to ¼ of the elution volume. Mix.

Ordering Information

For Ordering informtion please visit: www.biotage.com

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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Literature Number:PPS609

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