IMAC PhyTip® Columns

PhyTip® Columns

This specification sheet provides details on IMAC PhyTip® columns (Immobilized Metal Affinity Chromatography) affinity 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. IMAC PhyTip® columns are packed with Ni-IMAC affinity resin for purificaiton of histidine-tagged proteins.

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.

IMAC 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	70 µg
10 µL	130 µg
20 µL	270 µg
40 µL	530 µg
80 µL	1070 µg
160 µL	2130 µg
320 µL	4270 µg

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

IMAC Equilibration and Capture Buffer is labeled and supplied as a 5X concentrated solution of Phosphate Buffer containing 25 mM imidazole. We suggest having minimal imidazole in equilibration and in your sample to prevent non-specific binding and increase target yield but this is optional. To use as an equilibration buffer, dilute 5 X to a final concentration of 5 mM imidazole. We also recommend adding this buffer to your sample to a final concentration of 1X.

IMAC Wash Buffer 1 is labeled and supplied as a concentrated solution of Phosphate Buffer containing 100 mM imidazole. Recommended procedure is to dilute the buffer 20 X (5 mM imidazole) for use in the purification process, and higher concentrations should be evaluated for their effect on final purity. By varying the dilution of this buffer from 20 X to 1 X, purity and yield of final product may change. We recommend you begin with the lowest concentration of buffer and increase as needed to obtain the desired purity. As you increase the concentration of the Wash buffer there may be a reduction in yield of target protein.

IMAC Elution Buffer, as supplied, contains: 10 mM NaH_2PO_4, 0.3M NaCl and 200 mM Imidazole, pH7.4.

1000+ IMAC PhyTip® Columns

For a 500 µL sample with 5 µg His-Tagged Fab containing 1 mg BSA, processed with a 10 µL PhyTip® column and using the conditions shown below, greater than 50% of the original His-Tagged Fab mass is recovered in the final sample volume. In addition, this recovered His-Tagged Fab is purified to over >95% purity as determined by SDS-PAGE with Coomassie detection.

Equilibrate:
1000 µL of Biotage IMAC Capture 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/minute.

Wash:
1000 µL of Biotage IMAC Wash Buffer I, passed over the resin bed for two cycles at a flow rate 500 µL/min followed by a second wash with the same buffer, passed over the resin bed for two cycles at a flow rate 500 µL/min.

Elute:
Elute the protein into solution with 30 µL of Biotage IMAC Elution Buffer, passed over the resin bed for four cycles at a flow rate of 500 µL/min.

200+ IMAC PhyTip® Columns

For a 200 µL sample with 5 µg His-Tagged Fab containing 1 mg BSA processed using a 5 µL PhyTip® column and the conditions shown below, greater than 50% of the original His-Tagged Fab mass is recovered in the final sample volume. In addition, this recovered His-Tagged is purified to over >95% purity as determined by SDS-PAGE with Coomassie detection.

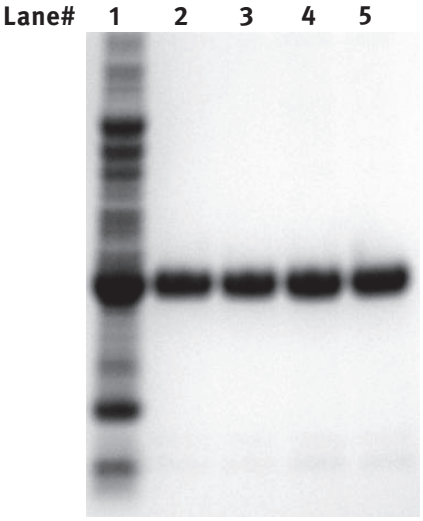
Equilibrate:
200 µL of Biotage IMAC capture buffer, passed over the resin bed for two cycles at a flow rate 250 µL/min.

Capture:
200 µL sample captured by passing through the resin bed for four cycles at a flow rate of 250 µL/min.

Wash:
200 µL of Biotage IMAC wash buffer I, passed over the resin bed for two cycles at a flow rate of 250 µL/min followed by a second wash with same buffer, passed over the resin bed for two cycles at a flow rate of 250 µL/min.

Elute:
Elute the protein into solution with 15 µL of IMAC elution buffer, passed over the resin bed for four cycles at a flow rate of 250 µL/min.

NuPAGE 4–12% Bis-Tris gel with MES Running Buffer



Lane 1 Ladder, Lanes 2-5 His-Tagged Fab

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: PPS610