

ProPlus PhyTip® Columns (MabSelect™ Sure™, GE Healthcare)



PhyTip® Columns

This specification sheet provides details on ProPlus PhyTip® Columns (MabSelect™ Sure™, GE Healthcare).

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. ProPlus PhyTip® columns are PhyTip® columns packed with ProPlus affinity resin (MabSelect™ Sure™, GE Healthcare) for purification of antibodies.

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 ProPlus 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.

ProPlus PhyTip® Columns

Table 1. PhyTip® Column Binding Capacity

Resin bed volume	Recommended binding capacity for highest recovery by dual flow chromatography
5 µL	50 µg
10 µL	100 µg
20 µL	200 µg
40 µL	400 µg
80 µL	800 µg
160 µL	1600 µg
320 µL	3200 µg

ProPlus 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.

All ProPlus PhyTip® columns are supplied with recommended PhyTip® buffers including:

Equilibration Buffer:

Phosphate Buffer solution pH 7.4.

Capture Buffer:

Provided 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:

Saline solution. **Note:** no buffering capacity to ensure effective elution.

Elution Buffer:

For the final elution step. Phosphate Buffer solution pH 2.5.

Neutralization Buffer:

Tris Buffer solution pH 9.0.

Note: Elution buffer is supplied as a pH 2.5 Phosphate buffer solution, if protein to be purified requires less acidic elution conditions the buffer can be adjusted using the Neutralization buffer.

i.e. For a pH 2.8 elution buffer, take 1 mL of standard elution buffer (pH 2.5) and add 30 µL of 1 M Tris buffer standard neutralization buffer to obtain 1 mL of pH 2.8 elution buffer (**actual pH may vary depending upon volumetric accuracy**)

For a pH 3.0 elution buffer, take 1 mL of standard elution buffer (pH 2.5) and add 40 µL of 1 M Tris buffer standard neutralization buffer to obtain 1 mL of pH 3.0 elution buffer (**actual pH may vary depending upon volumetric accuracy**)

For the neutralization step add 25% v/v of the elution volume e.g. if the elution volume is 20 µL, add 5 µL of 1 M Tris neutralization buffer.

1000+ ProPlus PhyTip® Columns

For a 1000 µL sample with 5 µg mIgG₁ (Southern Biotech), up to 50% of the original IgG mass is recovered in the final sample volume when purified with 80µL PhyTip® ProPlus columns (Table 2).

Equilibration:

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

Capture:

1000 µL sample captured by passing through the resin bed for four cycles at a flow rate of 500 uL per minute.

Wash:

1000 µL of Biotage ProPlus Wash Buffer I, passed over the resin bed for two cycles at a flow rate 500 µL/min followed by a second wash with 1000 µL Wash Buffer II, passed over the resin bed for two cycles at a flow rate of 500 µL/min. It is essential to use Wash Buffer II as it removes the pH 7.4 buffer from Wash I and in doing so ensures effective low pH elution during the elution step.

Elute:

Elute the protein into solution with 240 µL of Elution Buffer, passed over the resin bed for four cycles at a flow rate of 500 µL/min. Neutralize with 60 µL of Biotage Pro Plus Neutralization Buffer.

Table 2. mIgG₁ Recovery Using ProPlus PhyTip® Columns of Different Bed Volume

	20 µL	80 µL
%recovery	18.9	50.1
SD	1.1	2.2
% SD	5.8	6.0

200+ PhyTip® ProPlus columns

For a 200 µL sample with 1 µg mIgG₁ (Southern Biotech), up to 13% of the original IgG mass is recovered in the final sample volume when purified with 5 µL ProPlus PhyTip® columns (Table 3).

Equilibrate:

200 µL 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 250 µL per minute.

Wash:

200 µL of Biotage ProPlus Wash Buffer I, passed over the resin bed for two cycles at a flow rate 250 µL/min followed by a second wash with 200 µL Wash Buffer II, passed over the resin bed for two cycles at a flow rate of 250 µL/min. It is essential to use Wash Buffer II as it removes the pH 7.4 buffer from Wash I and in doing so ensures effective low pH elution during the elution step.

Elution:

Elute the protein into solution with 15 µL of Biotage ProPlus Elution Buffer, passed over the resin bed for four cycles at a flow rate of 250 µL/min. neutralize with 3.75 µL of Biotage Pro Plus neutralization buffer.

Table 3. mIgG₁ Recovery Using 200+ ProPlus PhyTip® Columns Containing 5 µL of Resin

	5 µL
%recovery	13.0
SD	0.8
% SD	5.9

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

Ordering Information

For Ordering information please visit: www.biotage.com

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