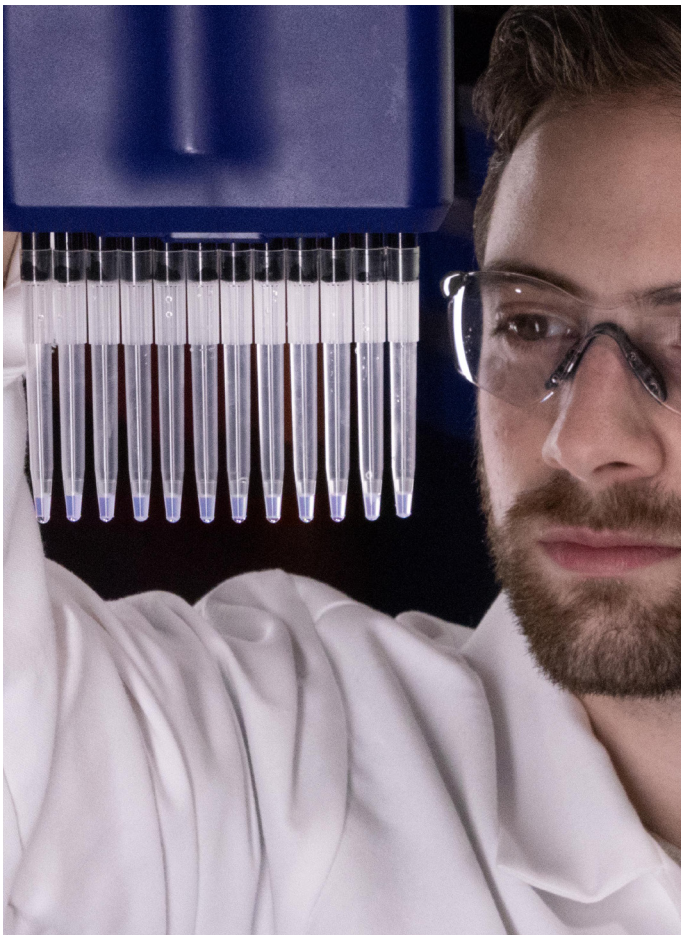
Protein G PhyTip® Columns



PhyTip® Columns

This specification sheet provides details on Protein G PhyTip®.

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. Protein G PhyTip® columns are packed with Protein G affinity resin for purification of IgG 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 Protein G 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).

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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.

Protein G PhyTip® Columns

Tabe 1. PhyTip® Column Binding Capacity

Resin bed volume	Recommended protein binding capacity for highest recovery by dual flow chromatography
5 µL	30 µg
10 µL	60 µg
20 µL	130 µg
40 µL	250 µg
80 µL	510 µg
160 µL	1010 µg
320 µL	2030 µg

Protein G 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.

A Buffer kit can be purchased together with Protein G PhyTip® columns. The buffer kit comes in different sizes and includes:

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 I Buffer:
Phosphate Buffer solution pH 7.4.

Wash II Buffer:
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+ Protein G PhyTip® Columns

For a 500 µL sample with 10 µg IgG2a (anti-FITC MAb.) containing 5 mg BSA, processed with a 10 µL PhyTip® column and using the conditions shown below, greater than 40% of the original IgG mass is recovered in the final sample volume. In addition, the recovered IgG is over 95% pure as determined by SDS-PAGE with Coomassie detection.

Equilibration:
1000 µL of Biotage Pro G 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/min.

Wash:
1000 µL of Biotage Protein G wash buffer I, passed over the resin bed for two cycles at a flow rate 500 µL/min followed by a second wash with wash buffer II, 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 Protein G elution buffer, passed over the resin bed for four cycles at a flow rate of 500 µL/min. neutralize with 5 µL of Biotage Protein G neutralization buffer.

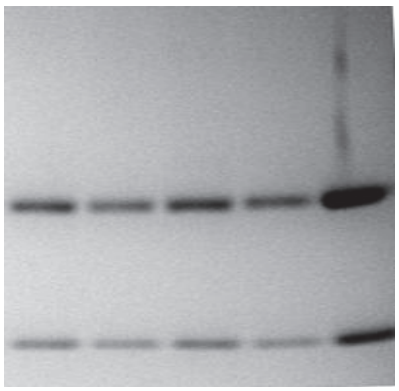
200+ Protein G PhyTip® Columns with Resin

For a 200 µL sample with 5 µg IgG2a (anti-FITC MAb.) containing 1 mg BSA, processed with a 5 µL PhyTip® column and using the conditions shown below, greater than 40% of the original IgG mass is recovered in the final sample volume. In addition, the recovered IgG is over 95% pure as determined by SDS-PAGE with Coomassie detection.

Equilibrate:
200 µL of Biotage Pro G 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 Protein G wash buffer I, passed over the resin bed for two cycles at a flow rate of 500 µL/min followed by a second wash with wash buffer II, passed over the resin bed for two cycles at a flow rate of 250 µL/min.



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

Lane	1	2	3	4	5

Lane	
1	2 µL IgG2a from PBS + 5 mg BSA
2	2 µL IgG2a from PBS
3	2 µL IgG2a from PBS + 5 mg BSA
4	2 µL IgG2a from PBS
5	2 µL unprocessed IgG2a

Elution:
Elute the protein into solution with 15 µL of Biotage Protein G elution buffer, passed over the resin bed for four cycles at a flow rate of 250 µL/min. Neutralize with 3 µL of Biotage Protein G neutralization buffer.

Species	Subclass	Protein G Binding
Human	IgA	-
	IgD	-
	IgE	
	IgG_1	++++
	IgG_2	++++
	IgG_3	++++
	IgG_4	++++
	IgM	-
Chicken	IgY	-
Avian egg yolk	IgY	-
Cow		++++
Dog		+
Goat		++
Guinea pig	IgG_1	++
	IgG_2	++
Hamster		++
Horse		++++
Monkey		++++
Mouse	IgG_1	++++
	$IgG2_a$	++++
	IgG2b	+++
	IgG_3	+++
	IgM_1	-
Pig		+++
Rabbit		+++
Rat	IgG_1	+
	$IgG2_a$	++++
	$IgG2_b$	++
	IgG_3	++
Sheep		++

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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