

Glutathione PhyTip® Columns



PhyTip® Columns

This specification sheet provides details on Glutathione 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. Glutathione PhyTip® columns are PhyTip® columns packed with Glutathione affinity resin for purification of tagget proteins.

Biotage recommends the use of free GST or similar 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.

Glutathione 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.

Glutathione PhyTip® Columns

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

Note that flow rate is of critical importance when performing glutathione-based separations; slower flow rates are typically required as compared to other resins.

1000+ Glutathione PhyTip® Columns

A 500 µL *E. coli* lysate sample with 15 µg GST was processed using the conditions shown below. Greater than 20% of the original GST mass is recovered in the final sample volume. In addition, this recovered GST is purified to >95% purity as determined by HPLC with UV absorbance detection at 215 nm.

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 is processed by passing through the resin bed for two cycles at 60 µL/minute.

Wash:

1000 µL of Glutathione Wash Buffer (typically 1xPBS) is passed through the resin bed for two cycles at 500 µL/min, followed by a second wash with the same buffer passed through the resin bed for two cycles at 500 µL/min.

Elute:

Elute the protein into solution with 15 µL of Glutathione Elution Buffer (50 mM Tris pH 8.0, 10mM glutathione) passed through the resin bed for four cycles at a flow rate of 500 µL/min.

200+ Glutathione PhyTip® Columns

A 200 µL *E. coli* lysate sample with 6 µg GST was processed using the conditions shown below. Greater than 20% of the original GST mass is recovered in the final sample volume. In addition, this recovered GST is purified to >95% purity as determined by HPLC with UV absorbance detection at 215 nm.

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 is processed by passing through the resin bed for two cycles at 60 µL/minute.

Wash:

200 µL of Glutathione Wash Buffer (typically 1xPBS) is passed through the resin bed for two cycles at 500 µL/min, followed by a second wash with the same buffer passed through the resin bed for two cycles at 500 µL/min.

Elute:

Elute the protein into solution with 10 µL of Glutathione Elution Buffer (50 mM Tris pH 8.0, 10mM glutathione) passed through the resin bed for four cycles at a flow rate of 500 µL/min.

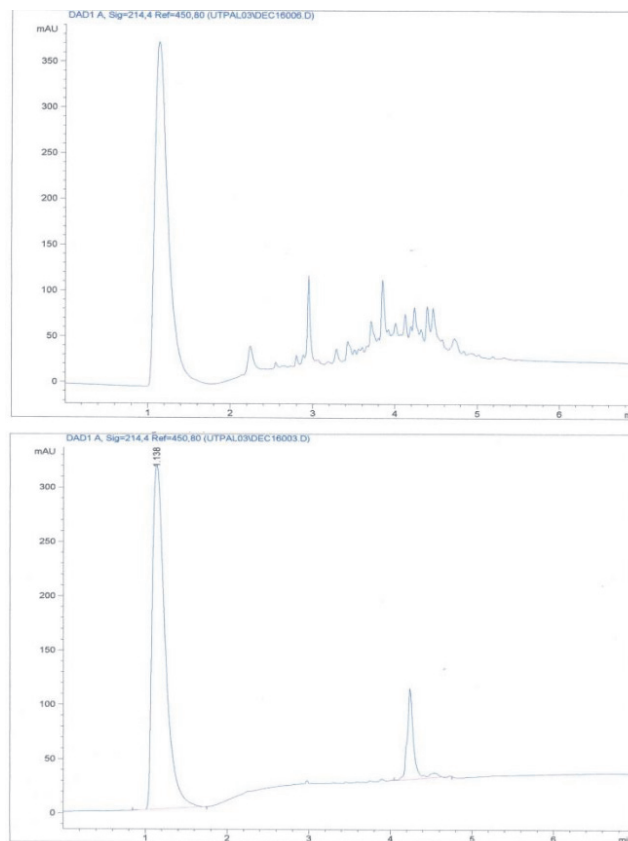


Figure 1: Top: HPLC of 200 µL unprocessed *E. coli* lysate + 6 µg GST
Bottom: HPLC of processed *E. coli* lysate.

Ordering Information

For Ordering information please visit: www.biotage.com

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7,875,462; 7,943,393; 8,057,668; 8,148,168

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