



PhyNexus, Inc.

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PhyNexus PhyTip® Columns with glutathione affinity resin

Performance Information Sheet.

This specification sheet provides details on PhyTip Columns containing Glutathione as the affinity resin.

PhyTip columns are unique capture, purification and enrichment tools from PhyNexus 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.

Samples for purification and enrichment must be clear and free from particulate matter. It is highly recommended to centrifuge samples and remove the supernatant only, prior to use with PhyTip columns.

PhyTip Columns

PhyTip columns are available in two formats, 200+ with a maximum sample volume of 200 μ L and 1000+ with a maximum volume of 1000 μ L. For each 200+ PhyTip column the volume of glutathione resin bed is 5 μ L and for the 1000+ PhyTip columns the resin bed is 10 μ L, where the resin is bound by a retention mesh that reduces dead space to a minimum. Integrated design of the PhyTip column and resin bed insure maximum capture potential and protein elution for the affinity resin. 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 have been manufactured 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 stored in Glycerol when shipped from PhyNexus.

Glutathione PhyTip columns

Glutathione PhyTip columns have been optimized for use with specific PhyNexus reagents and instrument flow rates/volumes as shown below. This information was collected using the PhyTip ME 1000 and ME 200 Purification Systems.

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+ PhyTip columns with Glutathione resin:

A 500 μ L E. coli lysate sample with 15 μ g 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.

Capture: 500 μ L sample is processed by passing through the resin bed for two cycles at 60 μ L/minute.

Purify: 1000 μ L of PhyNexus Glutathione Wash Buffer I is passed through the resin bed for two cycles at 500 μ L/min, followed by a second wash with the same buffer passed through the resin bed for two cycles at 500 μ L/min.

Enrich: elute the protein into solution with 15 μ L of PhyNexus Glutathione Elution Buffer passed through the resin bed for four cycles at a flow rate of 500 μ L/min.

200+ PhyTip columns with Glutathione resin:

A 200 μ L E. coli lysate sample with 6 μ g 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.

Capture: 200 μ L sample is processed by passing through the resin bed for two cycles at 60 μ L/minute.

Purify: 200 μ L of PhyNexus Glutathione Wash Buffer I is passed through the resin bed for two cycles at 500 μ L/min, followed by a second wash with the same buffer passed through the resin bed for two cycles at 500 μ L/min.

Enrich: elute the protein into solution with 10 μ L of PhyNexus Glutathione Elution Buffer passed through the resin bed for four cycles at a flow rate of 500 μ L/min.

Protocols for Capture, Purification and Enrichment of protein sample

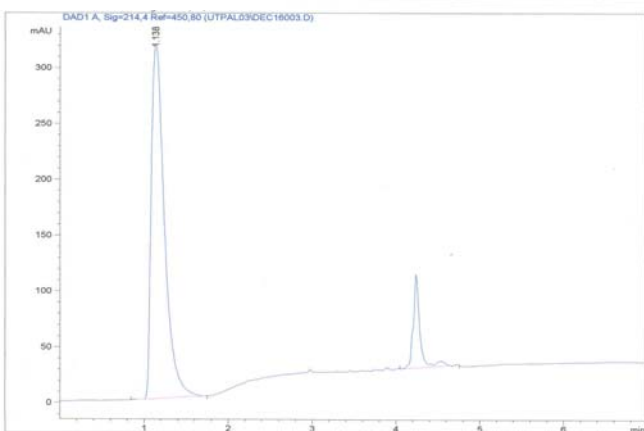
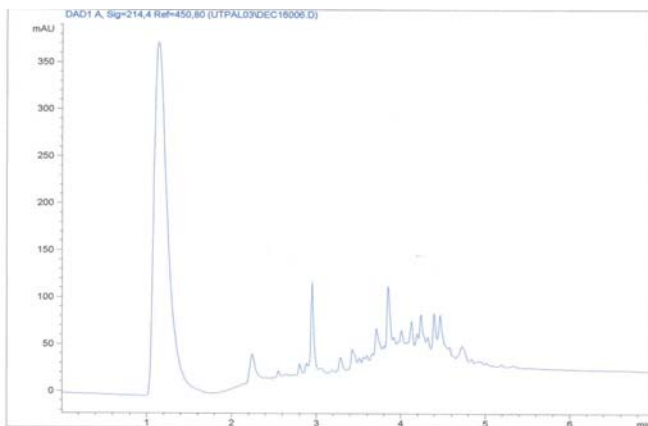
Using the PhyTip ME 1000 and ME 200 Purification Systems

Follow the built in methods and pop up instructions for Glutathione as indicated when using the computer controlled ME 1000 and ME 200 Purification Systems.

For further support call PhyNexus at 408-267-7214 or e-mail support@phynexus.com.

For further information on PhyNexus products, visit our website at www.phynexus.com.

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Top: HPLC of 200 μ L unprocessed E. coli lysate + 6 μ g GST
Bottom: HPLC of processed E. coli lysate