



PhyNexus, Inc.

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PhyNexus PhyTip® Columns with IMAC affinity resin

Performance Information Sheet.

This specification sheet provides details on PhyTip Columns containing IMAC (Immobilized Metal Affinity Chromatography) 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 use the clear 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 µL and 1000+ with a maximum volume of 1000 µL. For each 200+ PhyTip column, the volume of IMAC resin bed is 5 µL, 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. For the 1000+ PhyTip columns, the resin bed is 10 µL. 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.

IMAC PhyTip columns are stored in Glycerol when shipped from PhyNexus.

IMAC PhyTip columns

IMAC 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.

IMAC Wash Buffer I is labeled and supplied as a concentrated solution of Phosphate Buffer containing Imidazole. Recommended procedure is to dilute the buffer 20 X 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 NaH₂PO₄, 0.3M NaCl and 200 mM Imidazole, pH7.4.

1000+ PhyTip columns with IMAC resin:

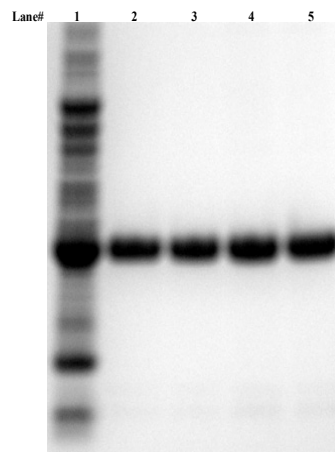
For a 500 μ L sample with 5 μ g His-Tagged Fab containing 1 mg BSA, processed using the conditions shown below, greater than 50% of the original His-Tagged Fab mass is recovered in the final sample volume. In addition, this recovered His-Tagged Fab is purified to over >95% purity as determined by SDS-PAGE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

Capture: 500 μ L sample captured by passing through the resin bed for two cycles at a flow rate of 250 μ L per minute.

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

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

NuPAGE 4-12% Bis-Tris gel with MES running buffer



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

200+ PhyTip columns with IMAC resin:

For a 200 μ L sample with 5 μ g His-Tagged Fab containing 1 mg BSA processed 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 is purified to over >95% purity as determined by SDS-PAGE with Coomassie detection.

Capture: 200 μ L sample captured by passing through the resin bed for two cycles at a flow rate of 250 μ L per minute.

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

Enrich: elute the protein into solution with 10 μ L of IMAC Elution Buffer, passed over the resin bed for five 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 IMAC 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.