



# PhyNexus, Inc.

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## PhyNexus PhyTip® ProPlus™ Columns for Antibody Affinity Purification

### Performance information sheet.

*This specification sheet provides details on PhyTip ProPlus Columns*

PhyTip columns are unique capture, purification and enrichment tools from PhyNexus designed for small 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 recommended maximum sample volume of 200  $\mu$ L and 1000+ with a recommended maximum volume of 1000  $\mu$ L. For each of the PhyTip column formats there are a number of 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 QC'd 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 ProPlus columns are stored in Glycerol when shipped from PhyNexus.

### PhyTip ProPlus columns

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

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

*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 so as to ensure effective elution

*Enrichment Buffer* – for the final elution step – Phosphate Buffer solution pH 2.5

*Neutralization Buffer.* – Tris Buffer solution pH **9.0**

Note: Enrichment buffer is supplied as 4 mL of a pH 2.5 Phosphate buffer solution, if protein to be purified requires less acidic elution conditions e.g. pH 2.8, the enrichment buffer pH can be changed as follows: Take 1 mL of standard Enrichment Buffer (pH 2.5) and add **30  $\mu$ L** 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 enrichment buffer, take 1 mL of standard Enrichment Buffer (pH 2.5) and add **40  $\mu$ L** 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  $\mu$ L add 5  $\mu$ L of 1 M Tris Neutralization Buffer.

### 1000+ PhyTip ProPlus columns:

For a 1000  $\mu\text{L}$  sample with 5  $\mu\text{g}$  mlgG<sub>1</sub> (Southern Biotech), up to 50% of the original IgG mass is recovered in the final sample volume when purified with 80 $\mu\text{L}$  PhyTip ProPlus columns (Table 1).

*Capture:* 1000  $\mu\text{L}$  sample captured by passing through the resin bed for four cycles at a flow rate of 250  $\mu\text{L}$  per minute.

*Purify:* 1000  $\mu\text{L}$  of PhyNexus ProPlus Wash Buffer I, passed over the resin bed for one cycle at a flow rate 500  $\mu\text{L}/\text{min}$  followed by a second wash with 1000  $\mu\text{L}$  Wash Buffer II, passed over the resin bed for one cycle at a flow rate of 500  $\mu\text{L}/\text{min}$ . 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 enrichment step.

*Enrich:* elute the protein into solution with 240  $\mu\text{L}$  of PhyNexus ProPlus Enrichment Buffer, passed over the resin bed for four cycles at a flow rate of 250  $\mu\text{L}/\text{min}$ . Neutralize with 60  $\mu\text{L}$  of PhyNexus Protein A Neutralization Buffer.

**Table 1: mlgG<sub>1</sub> recovery using PhyTip ProPlus columns of different bed volume**

	20 $\mu\text{L}$	80 $\mu\text{L}$
%recovery	18.9	50.1
SD	1.1	2.2
% SD	5.8	6.0

### 200+ PhyTip ProPlus columns:

For a 200  $\mu\text{L}$  sample with 1  $\mu\text{g}$  mlgG<sub>1</sub> (Southern Biotech), up to 13% of the original IgG mass is recovered in the final sample volume when purified with 5  $\mu\text{L}$  PhyTip ProPlus columns (Table 2).

*Capture:* 200  $\mu\text{L}$  sample captured by passing through the resin bed for four cycles at a flow rate of 100  $\mu\text{L}$  per minute.

*Purify:* 200  $\mu\text{L}$  of PhyNexus ProPlus Wash Buffer I, passed over the resin bed for one cycle at a flow rate 250  $\mu\text{L}/\text{min}$  followed by a second wash with 200  $\mu\text{L}$  Wash Buffer II, passed over the resin bed for one cycle at a flow rate of 250  $\mu\text{L}/\text{min}$ . 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 enrichment step.

*Enrich:* elute the protein into solution with 15  $\mu\text{L}$  of PhyNexus ProPlus Enrichment Buffer, passed over the resin bed for four cycles at a flow rate of 250  $\mu\text{L}/\text{min}$ . Neutralize with 3.75  $\mu\text{L}$  of PhyNexus Protein A Neutralization Buffer.

**Table 2: mlgG<sub>1</sub> recovery using 200+ PhyTip ProPlus columns containing 5 $\mu\text{L}$  of resin**

%recovery	13.0
SD	0.8
% SD	5.9

### Protocols for Capture, Purification and Enrichment of protein sample

#### Using the PhyTip MEA Personal Purification System, ME 1000 and ME 200 Purification Systems

Follow the built in methods and pop up instructions for PhyTip ProPlus columns as indicated when using the computer controlled MEA Personal Purification System, ME 1000 and ME 200 Purification Systems.

For further support, call PhyNexus at 408-267-7214, e-mail [support@phynexus.com](mailto:support@phynexus.com), or visit our website at [www.phynexus.com](http://www.phynexus.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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