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PhyNexus PhyTip® Columns with Protein A affinity resin

Performance information sheet.

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

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 µL and 1000+ with a recommended maximum volume of 1000 µL. 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 columns with Protein A are stored in Glycerol when shipped from PhyNexus.

PhyTip columns with Protein A

PhyTip columns with Protein 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 columns with Protein 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 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 enrichment buffer, take 1 mL of standard Enrichment 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+ *PhyTip* columns with Protein A resin:

For a 500 μ L sample with 5 μ g mIgG2a (Sigma-Aldrich), containing 1 mg BSA processed using the conditions shown below, up to 94% 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 (Fig.1).

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

Purify: 500 μ L of *PhyNexus* Protein A Wash Buffer I, passed over the resin bed for one cycle at a flow rate 250 μ L/min followed by a second wash with one mL Wash Buffer II, passed over the resin bed for cycle at a flow rate of 250 μ L/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 μ L of *PhyNexus* Protein A Enrichment Buffer, passed over the resin bed for four cycles at a flow rate of 250 μ L/min. Neutralize with 5 μ L of *PhyNexus* Protein A Neutralization Buffer.

Example of IgG purification with Protein A *PhyTip* columns

Nu-PAGE 4-12% Bis-Tris gel with MES running buffer

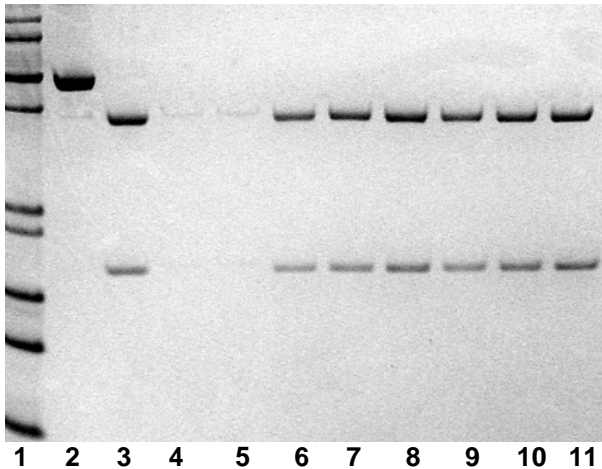


Fig.1: Lane 1: marker, 2: 2 μ g BSA, 3: 2 μ g IgG_{2a}, 4, 5: Protein A resin only; 6, 7, 8: 2 μ L each of Protein A purified IgG_{2a} from PBS, PBS containing 5 mg BSA (2 and 5 cycles loading), respectively; 9, 10, 11: 2 μ L each of Protein A purified IgG_{2a} from PBS, PBS containing 5 mg BSA (2 and 5 cycles loading), respectively.

200+ *PhyTip* columns with Protein A resin:

For a 200 μ L sample with 5 μ g mIgG2a (Sigma-Aldrich) containing 1 mg BSA, processed using the conditions shown below, greater than 50% 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 (Fig.1).

Capture: 200 μ L sample captured by passing through the resin bed for four cycles at a flow rate of 100 μ L per minute.

Purify: 100 μ L of *PhyNexus* Protein A Wash Buffer I, passed over the resin bed for one cycle at a flow rate of 250 μ L/min followed by a second wash with 200 μ L Wash Buffer II, passed over the resin bed for one cycle at a flow rate of 250 μ L/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 μ L of *PhyNexus* Protein A Enrichment Buffer, passed over the resin bed for four cycles at a flow rate of 100 μ L/min. Neutralize with 3 μ L of *PhyNexus* Protein A Neutralization Buffer.

- Enrichment Buffer components include: 200mM NaH₂PO₄, 140mM NaCl @ pH 2.5

Capacity of *PhyTip* columns with Protein A resin:

Miniaturizing bed volumes in the *PhyTip* column format shows no adverse effects. Purification of 15 mg/mL polyclonal human IgG samples (Sigma-Aldrich) typically results in recovery of 29 μ g hIgG per μ L Protein A resin for all column formats (Fig.2A). Under dilute starting sample concentrations of 5 μ g mIgG2a (Sigma-Aldrich) spiked into 500 μ L capture buffer, recovery increases with increasing resin bed volume (Fig. 2B). Typically, use of a column format is dependent upon a user's final sample concentration needs (Fig. 3C). An elution volume of 3 times the resin bed volume is recommended.

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 Protein A as indicated when using the computer controlled MEA Personal Purification System, ME 1000 and ME 200 Purification Systems.

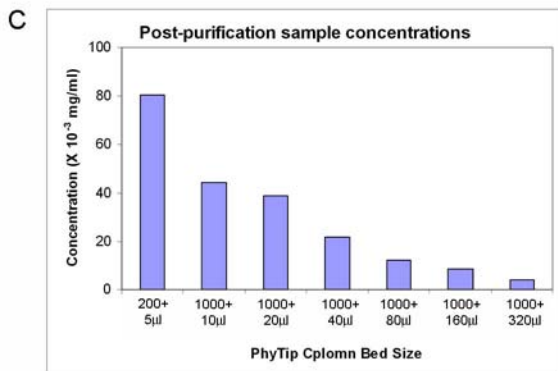
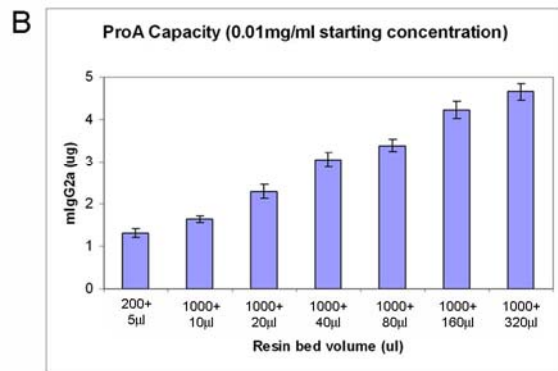
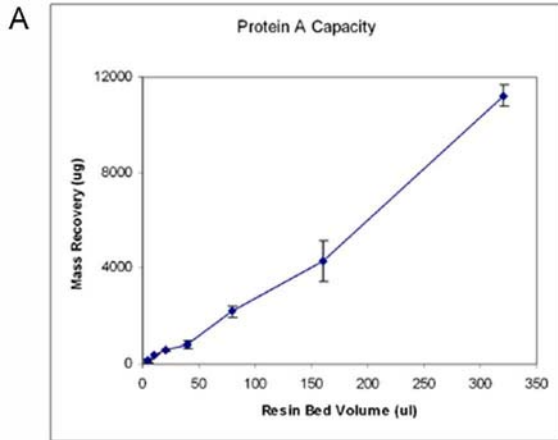


Fig. 2: Capacity of PhyTip columns in available bed volume formats.

Protein A has been demonstrated to have the following binding affinity.

Species	Subclass	Protein A Binding	
Human	IgA	variable	
	IgD	-	
	IgE	-	
	IgG ₁	++++	
	IgG ₂	++++	
	IgG ₃	-	
	IgG ₄	++++	
	IgM	variable	
	Chicken	IgY	-
	Avian egg yolk	IgY	-
Cow		++	
Dog		++	
Goat		-	
Guinea pig	IgG ₁	++++	
	IgG ₂	++++	
Hamster		+	
Horse		++	
Monkey		++++	
Mouse	IgG ₁	+	
	IgG _{2a}	++++	
	IgG _{2b}	+++	
	IgG ₃	++	
	IgM ₁	variable	
Pig		+++	
Rabbit	no distinction	++++	
Rat	IgG ₁	-	
	IgG _{2a}	-	
	IgG _{2b}	-	
	IgG ₃	+	
Sheep		+/-	

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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