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PhyNexus PhyTip® Columns with Protein G affinity resin

Performance Information Sheet.

This specification sheet provides details on PhyTip Columns containing Protein G 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.

PhyNexus recommends the use of the PhyTip Starter Kit containing IgG as a 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

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 G are stored in Glycerol when shipped from PhyNexus.

PhyTip columns with Protein G

PhyTip columns with Protein G 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

All PhyTip columns with Protein G 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 G resin:

For a 500 μ L sample with 10 μ g IgG2a (anti-FITC MAb.) containing 5 mg BSA, processed using the conditions shown below, greater than 40% 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.

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: 1000 μ L of PhyNexus Protein G 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 Wash Buffer II, 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 Protein G Enrichment Buffer, passed over the resin bed for five cycles at a flow rate of 500 μ L/min. Neutralize with 5 μ L of PhyNexus Protein G Neutralization Buffer.

200+ PhyTip columns with Protein G resin:

For a 200 μ L sample with 5 μ g IgG2a (anti-FITC MAb.) containing 1 mg BSA, processed using the conditions shown below, greater than 40% 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.

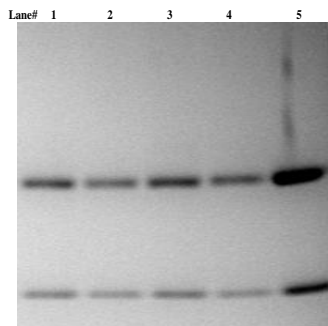
Capture: 200 μ L sample captured by passing through the resin bed for four cycles at a flow rate of 250 μ L per minute.

Purify: 200 μ L of PhyNexus Protein G 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 Wash Buffer II, 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 PhyNexus Protein G Enrichment Buffer, passed over the resin bed for five cycles at a flow rate of 500 μ L/min. Neutralize with 3 μ L of PhyNexus Protein G Neutralization Buffer.

Protein G Enrichment Buffer as shipped contains: 111mM NaH_2PO_4 , 140mM NaCl in 14.8mM H_3PO_4 , pH 3.0

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



Ln1: 2 ul IgG2a from PBS+5 mg BSA
Ln2: 2 ul IgG2a from PBS
Ln3: 2 ul IgG2a from PBS+5 mg BSA
Ln4: 2 ul IgG2a from PBS
Ln5: 2 ug unprocessed IgG2a

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

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

Species	Subclass	Protein G Binding
Human	IgA	-
	IgD	-
	IgE	
	IgG ₁	++++
	IgG ₂	++++
	IgG ₃	++++
	IgG ₄	++++
	IgM	-
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	-
Pig		+++
Rabbit		+++
Rat	IgG ₁	+
	IgG _{2a}	++++
	IgG _{2b}	++
	IgG ₃	++
Sheep		++

For further support, call PhyNexus at 408-267-7214,
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www.phynexus.com

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7,837,871; 7,875,462; 7,943,393; 8,057,668; 8,148,168

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