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PhyNexus PhyTip® Columns with Streptavidin affinity resin

Performance information sheet.

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

PhyTip columns are unique capture, purification and enrichment™ tools from PhyNexus designed for lab-scale 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 biotin-labeled bovine albumin (Sigma, A8549) 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 maximum sample volume of 200 µL and 1000+ with a maximum volume of 1000 µL. For each 200+ PhyTip column, the volume of glutathione resin bed is 5 µL or 10 µL and for the 1000+ PhyTip columns the resin bed is 10 µL, 20 µL, 40 µL, 80 µL 160 µL or 320 µL, 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 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 Streptavidin are stored in Glycerol when shipped from PhyNexus.

PhyTip columns with Streptavidin

PhyTip columns with Streptavidin have been optimized for use with general molecular biology laboratory reagents and specific PhyNexus instrument flow rates/volumes as shown below. This information was collected using the PhyNexus Purification Systems.

PhyNexus recommends the following buffers (not supplied):
Capture Buffer – Phosphate Buffer solution pH 7.4, 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 – Phosphate Buffer solution pH 7.4
Enrichment Buffer – If performing a pull-down assay, for the final elution step may be compatible with an acidic buffer such as Phosphate Buffer solution pH 2.5
Neutralization Buffer. – Tris Buffer solution pH **9.0**

***1000+ PhyTip columns with Streptavidin resin**:*

For a 500 μL sample with 10 μg biotin-albumin spiked into PBS buffer and processed using the conditions shown below, greater than 80% of the original biotin-albumin mass is immobilized.

Capture: 500 μL sample captured by passing through the resin bed for four cycles at a flow rate of 250 uL per minute. A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip column at 1-2 mm above the bottom of the well.

Purify: 1000 μL of PBS, passed over the resin bed for two cycles at a flow rate 500 μL/min. A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip column at 1-2 mm above the bottom of the well.

Affinity Pull-Down: 500 μL sample captured by passing through the resin bed for four to eight cycles at a flow rate of 250 uL per minute. A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip column at 1-2 mm above the bottom of the well.

Enrich: Elute the protein into solution with a voliume of three times the resin bed volume of Enrichment Buffer, passed over the resin bed for four cycles at a flow rate of 500 μL/min. A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip column at 1-2 mm above the bottom of the well.

Neutralize: Neutralize with1M Tris pH 9.0 using a volume equal to ¼ of the elution volume. Mix.

200+ PhyTip columns with Streptavidin resin:

For a 200 μL sample with 10 μg biotin-albumin spiked into PBS buffer and processed using the conditions shown below, greater than 80% of the original biotin-albumin mass is immobilized.

Capture: 200 μL sample captured by passing through the resin bed for four cycles at a flow rate of 250 uL per minute. A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip column at 1-2 mm above the bottom of the well.

Purify: 200 μL of PBS, passed over the resin bed for two cycles at a flow rate 500 μL/min. A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip column at 1-2 mm above the bottom of the well.

Affinity Pull-Down: 200 μL sample captured by passing through the resin bed for four to eight cycles at a flow rate of 250 uL per minute. A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip column at 1-2 mm above the bottom of the well.

Enrich: Elute the protein into solution with a voliume of three times the resin bed volume of Enrichment Buffer, passed over the resin bed for four cycles at a flow rate of 500 μL/min. A cycle consists of aspirate, pause 20 seconds, dispense, pause 20 seconds all while maintaining the end of the PhyTip column at 1-2 mm above the bottom of the well.

Neutralize: Neutralize with1M Tris pH 9.0 using a volume equal to ¼ of the elution volume. Mix.

Protocols for Capture, Purification and Enrichment of protein sample

Using the PhyTip MEA 2 Personal Purification System

Follow the built in methods and pop up instructions for Streptavidin as indicated when using the computer controlled MEA 2 Personal Purification System.

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