PhyNexus

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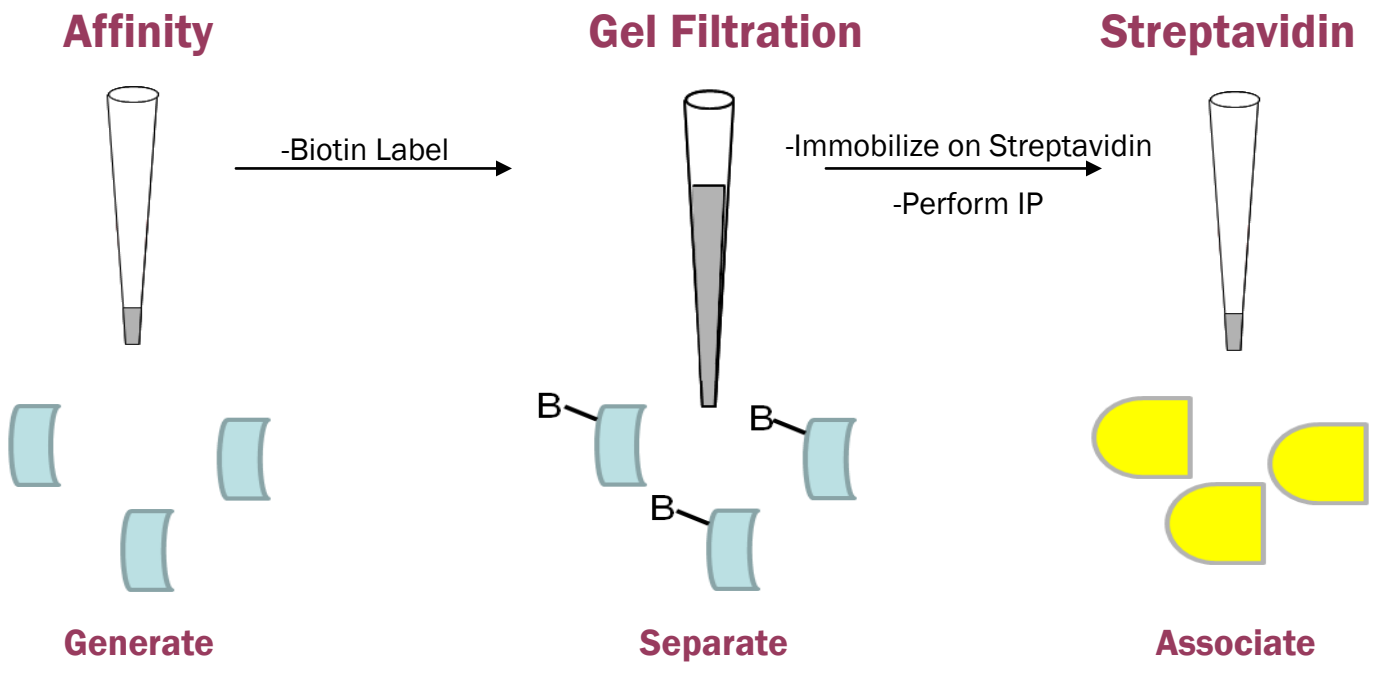
PHYTIP® COLUMNS FOR BIOTINYLATED PROTEIN ASSAYS

The challenge of modern Target Discovery requires the efficient automation of complex assays. The immunoprecipitation (IP) assay has proven quite useful in research discovery to identify components of molecular complexes, discovering protein-protein and protein-nucleic acid interactions, and quantifying an immunogenic response. Protein A PhyTip® columns have been successfully used for this purpose but IP can only be successful if an antibody is available to the peptide or protein of interest. The availability of biotin labeling reagents allows researchers to generate customized affinity resins by conjugating proteins of interest to streptavidin resins. These custom resins are then ready to pull down interactive proteins and complexes from biological samples. To efficiently automate these assays, significant sample preparation steps must be addressed. Namely, protein needs to be purified, excess biotin labeling reagent must be removed, and biotinylated protein needs to be immobilized. PhyNexus Affinity Purification, Gel Filtration columns containing resin of 5K molecular weight cutoff, and Streptavidin columns in combination are the ideal tools for small-volume, automated, high throughput purification, separation, and immobilization of biotinylated proteins for complex assays.

- Process minimal protein sample for highly purified starting protein
- Process small biotin labeling reaction volumes of 20 — 400 µL
- Run 1 to 96 samples manually or in a completely automated format
- Entire assay can be automated on same platform for consistency
- Use in conjunction with PhyTip® affinity, ion exchange, and streptavidin resins allows for a complete, automated, high-throughput immunoprecipitation platform



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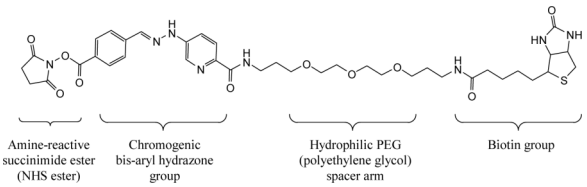
Example Immunoprecipitation Procedure

(1) Purify protein sample with PhyTip® Affinty column

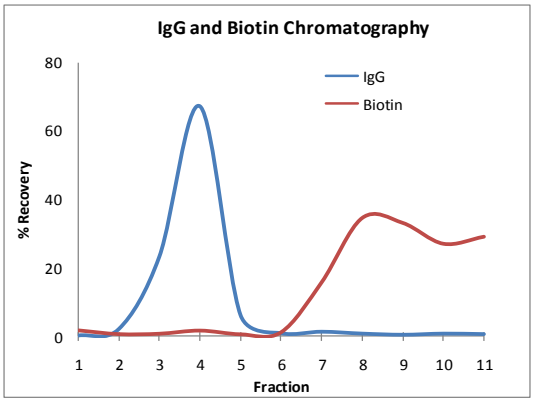
	Affinity Purification By PhyTip® Columns
% recovery	~80%*
% purity	>95%
Yield CV	<10

* The % recovery is highly dependent upon starting sample concentration.
High purity is required for accurate assessment of IP results.

(2) Label protein via available lysine residues with NHS-biotin reagent (Pierce, 21325)

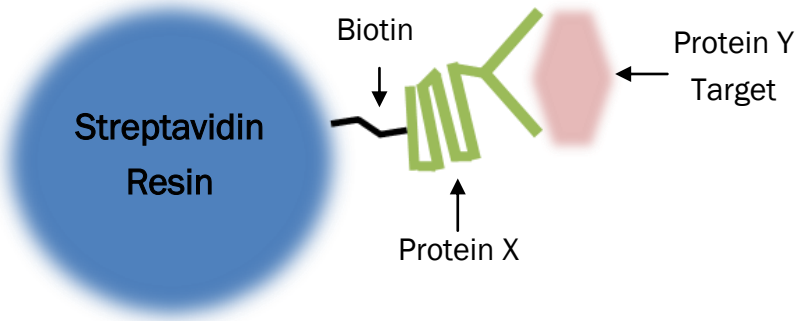


(3) Remove excess biotin labeling reagent with PhyTip® Gel Filtration Column



Protein and Biotin Chromatographic Profiles
20µL samples composed of either IgG (Sigma, 56834) or NHS-Chromogenic-Biotin (Pierce, 21325) were processed by separate PhyTip® columns containing 200µL 5K gel filtration media. The Flow Through and 10 fractions of 25µL, each, were collected and analyzed by the NanoDrop UV Spectrometer.

(4) Immobilize biotin-protein onto PhyTip® Streptavidin Columns and perform assay



IP Experiment: 5 mL Streptavidin PhyTip® columns immobilized with biotin-Protein X used to pull down Protein Y	
Protein Y Captured by biotin-Protein X	10 mg
Protein Y Recovered	8 mg
% of catured Protein Y recovered	90%