

Improved Process Efficiency with 96-well Protein Purification and Characterization

Jeremy Lambert, Murray Anderson, Christopher Hanna, PhyNexus, Inc., San Jose, CA
Lynn Jordan, Abbie L. Esterman, Seth Cohen, Caliper Life Sciences, Inc., Hopkinton, MA

Introduction

The majority of today's antibody therapeutic companies are driven by a need to effectively identify and characterize large collections of antibodies and proteins with the goal of decreasing time to market of protein therapeutics. Technologies that provide detailed information on protein structure, function, and activity have increased in throughput over the past several years and the trend is expected to continue, however until very recently solutions for high-throughput purification and enrichment of proteins have lagged behind. In this poster we present an integrated approach that includes the automated purification of micro volume protein samples in a 96-well format along with real time monitoring of the resulting purity and concentration data thus increasing the throughput capability and productivity, and ultimately enabling throughput of downstream applications on a scale that is no longer limited by sample preparation.

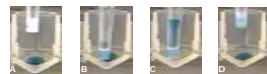
PhyTip™ Columns

PhyNexus has developed a unique, yet simple process to purify and enrich antibodies or recombinant proteins containing affinity tags. PhyTip columns can be used in applications ranging from simple low throughput to fully automated 96-well processes. These unique devices are capable of preparing concentrations of up to 5 mg/ml of purified protein in small final volumes e.g. 10-20 µl, in less than 15 minutes.

To demonstrate the effectiveness of this new sample preparation system, antibodies and recombinant affinity-tagged proteins were processed with PhyTip columns. These columns are specifically designed to contain micro-volume quantities of conventional affinity separation media in a manner that introduces virtually zero dead volume.



PhyTip columns encapsulate a small amount of resin within the end of a pipette tip. Columns are available in two sizes, 1000 µl and 200 µl, containing 10 and 5 microliters of affinity resin, respectively. The choice in column size provides flexibility in enrichment capacity and throughput.

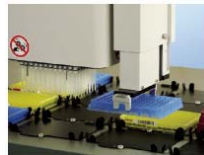


PhyTip Enrichment Process

The design of the columns allows for reproducible and predictable flow fluid through the micro-volume of affinity resin. The images above show a dye solution to illustrate the elution process within the tips. 10 µl of elution buffer is placed in the bottom of a 96 or 384 well plate (A). After loading and washing steps are complete, the elution buffer is passed repeatedly through the bed to maximize recovery from the column. The characteristics of the bed and screen design maintain an intact volume of elution buffer (B-D), allowing for high enrichment factors and reproducible performance.

Automated Enrichment with Sciclone ALH 3000

Murine IgG_{2a} was added to DMEM + 10% FBS to simulate hybridoma growth conditions for mAb production and subsequent Protein A PhyTip enrichment. 6X-His tagged ubiquitin and GST-tagged ubiquitin were added to *E. coli* whole cell lysate to simulate recombinant protein purification using Ni-NTA and glutathione PhyTips. 200 µl of each sample was placed into wells of a 96-well plate.



The ALH 3000 provides highly controlled reproducible flow rates for the 96 tip head. The Gripper removes and holds the lid from each plate.

Automated enrichment was performed using Caliper's Sciclone ALH 3000 with a High Volume Head (volumes up to 200 µl). PhyTip columns were loaded directly from a 96-tip box onto the mandrels of the ALH 3000 head.

The following method was used for enrichment:

	Flow rate (µl/s)	Volume (µl)	# Cycles
Capture	4	200	2-12
Wash 1	10	200	2
Wash 2	10	200	2
Enrich	4	10	4

Protein Characterization using the LabChip 90

Microfluidic Chip and Sample Preparation

- Protein samples are denatured and reduced using a high concentration of SDS and BME.
- The microfluidic chip is primed with a gel mixture.
- The chip and sample plate are loaded onto the instrument and the software is started.

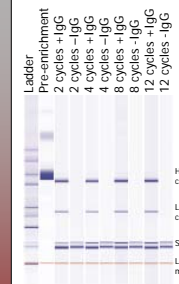


On-Chip Sample Processing

- A vacuum is applied at well A1 through the pressure load channel to aspirate sample from the well plate, through the sipper and onto the chip.
- Voltage is applied between wells 3 and 6 to electrokinetically inject a sample plug.
- Voltage is then applied between wells 3 and 5 to perform electrophoretic separation and staining.
- Electrokinetic de-staining and detection is achieved by diluting the separation channel contents from wells 2 and 7.



Optimization of IgG Enrichment



Virtual gel image of IgG enrichment as a function of capture cycles.

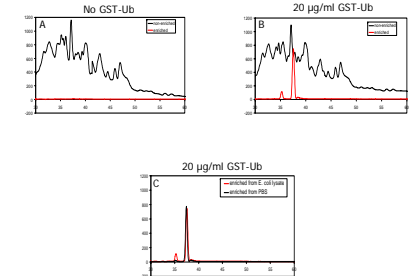
DMEM/FBS was spiked with +/- 20 µg/ml mouse IgG_{2a}. Samples were processed using increasing numbers of capture cycles (2-12). 5 µl of each sample were analyzed on the Caliper LC90. The enrichment factors are determined from the sum of the heavy and light chains as measured by the LC90 data regression software and are calculated relative to pure IgG_{2a} run under identical conditions on the LC90. The purity of the enriched material was greater than 95% for all conditions tested, with maximum recovery observed at 8 cycles.

# Cycles	Enrichment
2	10.7
4	11.4
8	12.7
12	12.3

GST Fusion Protein Enrichment

Electropherograms of GST-Ubiquitin enrichment.

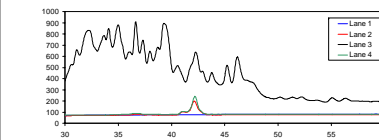
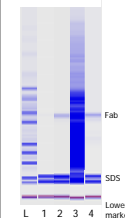
GST-Ubiquitin fusion protein was added to PBS and *E. coli* whole cell lysate at 20 µg/ml. The samples were enriched using Glutathione PhyTip columns. 8 capture cycles were used in the method on the Sciclone ALH 3000. Purified GST-Ub was eluted using 20 mM reduced glutathione. The samples were run on the LC90 under reducing conditions. Electropherograms below demonstrate the high purity of the enriched GST-Ub from a background of *E. coli* whole cell lysate (panels A and B). Panel C shows a comparison of GST-Ub enriched from PBS and enrichment from the lysate.



Ni-NTA IMAC Enrichment

Virtual gel image and electropherograms of 6X-tag Fab enrichment.

6XHis-tagged Fab was added to PBS and *E. coli* whole cell lysate at 5 µg/ml. The samples were enriched using Ni-NTA PhyTip columns. 8 capture cycles were used in the method on the Sciclone ALH 3000. Purified Fab was eluted using 500 mM imidazole. The samples were run on the LC90 using non-reducing conditions. Lane L = ladder, lane 1 = Fab in PBS pre-enrichment, lane 2 = Fab in PBS post-enrichment, lane 3 = Fab in *E. coli* lysate pre-enrichment, lane 4 = Fab in *E. coli* lysate post-enrichment. Electropherogram of the microfluidic analysis is shown below.



Summary

Automated selective enrichment of monoclonal antibodies from hybridoma growth media and recombinant 6X-His or GST-tagged fusion proteins from *E. coli* lysate using PhyTip columns on the Sciclone ALH 3000 workstation enables simultaneous purification and enrichment of up to 96 samples in under 15 minutes. Subsequent characterization (size, concentration, purity) of the enriched material using the LabChip 90 microfluidic protein analysis system provides a streamlined workflow and significant reduction in time and labor associated with high-throughput protein expression and purification. The purity and concentration of the final products enable downstream analytical and biochemical assays, increasing productivity and decreasing development time for therapeutic antibody drugs.

