A Novel Approach to Automated High-Throughput Protein Enrichment and Characterization

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

Introduction

The rapidly growing field of antibody and protein engineering continues to create the demand for technologies that increase the productivity and depth of the protein interaction analysis. In this poster, we describe a unique, simple process to purify and enrich antibodies or recombinant proteins containing affinity tags. PhyTip™ columns from PhyNexus are used in applications ranging from purifying-tagged-antibody to highly automated workflows. These columns are designed to concentrate and enrich proteins in small quantities, on a 96-well plate, in less than 15 minutes.

PhyTip™ Columns

PhyNexus has developed a unique, simple process to purify and enrich antibodies or recombinant proteins containing affinity tags. PhyTip™ columns from PhyNexus can be used in applications ranging from purifying-tagged antibodies to highly automated workflows. These columns are designed to concentrate and enrich proteins in small volumes, on a 96-well plate, in less than 15 minutes.

To demonstrate the effectiveness of this new workflow system, antibodies were purified with PhyTip™ columns. The process was designed to demonstrate the capability of a one-step, one-bottle purification of total antibodies. The antibody was then used to purify a total of 10 to 15 purged protein in small volumes up to 50 ml in less than 15 minutes.

PhyTip™ columns are available in a small volume of 100 µl, 300 µl, and 1.5 ml, containing 10 x 1 and 12 x 0.6 µl of antibody, respectively. The choice of volume is provided flexibility in enrichment capacity and throughput.

Automation with Scicome ALH 3000

A dilution series of the recombinant TllM protein was analyzed on a Scicome ALH 3000. The Scicome ALH 3000 is designed to perform high-throughput purification of recombinant proteins. Automation with Scicome ALH 3000 was performed using Caliper's Scicome ALH 3000 with a high-throughput automaton. The column was loaded with 10 µl of the protein solution at a flow rate of 100 µl/min. After loading, the column was washed with 10 µl of buffer at the same flow rate, and the flow rate was then increased to 100 µl/min to wash the column. The final concentration of the protein solution was then analyzed using the Human ELISA Kit to determine the concentration of the protein solution.

Results of Enrichment from DMEM/10% FBS

Virtual gel image of reduced hits sample before and after Protein A enrichment from DMEM/10% FBS. The left panel shows the gel image of the reduced hits sample before Protein A enrichment. The right panel shows the gel image of the reduced hits sample after Protein A enrichment. The arrows indicate the bands that were detected in the reduced hits sample before Protein A enrichment but not detected in the reduced hits sample after Protein A enrichment.

Results of Enrichment from PBS

Virtual gel image of reduced hits sample before and after Protein A enrichment from PBS. The left panel shows the gel image of the reduced hits sample before Protein A enrichment. The right panel shows the gel image of the reduced hits sample after Protein A enrichment. The arrows indicate the bands that were detected in the reduced hits sample before Protein A enrichment but not detected in the reduced hits sample after Protein A enrichment.

Summary

Automated depletion enrichment of recombinant antibodies from hybridoma growth media using PhyNexus PhyTip™ columns on the Scicome ALH 3000 workstation enables simultaneous parallel isolation of multiple samples within 10 minutes. This technology is designed to perform high-throughput purification of recombinant proteins. The workflow integrates automation and validated sample preparation steps to deliver a streamlined solution for rapid and robust antibody purification. The result is a significant reduction in time and labor required for high-throughput antibody purification and characterization.

LIC03-P05421