Comparison of Techniques for Small-Scale Purification of Protein

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Abstract

Baculovirus-mediated insect cell expression is frequently employed for the production of recombinant protein for structural and functional analysis. Prior to large-scale protein production, an optimisation of expression study is often undertaken to elucidate the most desirable constructs, and the conditions under which they express and purify. A typical study considers the effects of cell harvest time, volume of virus required, and cell line preferred. Here, we report findings from a recent evaluation of a number of high performance applications for the small-scale purification of histidine-tagged proteins.

Sample Preparation

Insect cells in 4ml suspension cultures (1.5 x 10^6 cells/ml) were infected with recombinant baculovirus at an MOI of 2 p.f.u/ml. Cultures were incubated at 27°C in a shaker incubator for 48hrs. Cells were harvested by centrifugation and samples prepared for purification. Pellets were re-suspended and the lysates were cleared by ultracentrifugation at 430,000g for 10 minutes at 4°C. Supernatants were then loaded onto the respective instruments. Six kinases (seven using magnetic bead-based technology) have been evaluated on the different systems with yields in the range 5mg/L to 20mg/L (final purified yield).

His Spin Columns

His Spin columns are pre-packed, single-use spin columns for purifying histidine-tagged proteins by Immobilised Metal Affinity Chromatography. The columns are packed with Nickel (Ni) Sepharose and used with a standard microcentrifuge.

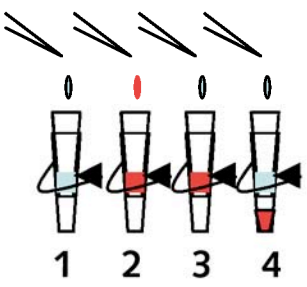

Figure 1: His Spin columns; purification commences in 4 easy steps. The column is equilibrated in binding buffer (1) before the sample is loaded (2). The column is then washed (3) and the protein elutes with the addition of 500mM Imidazole (4).

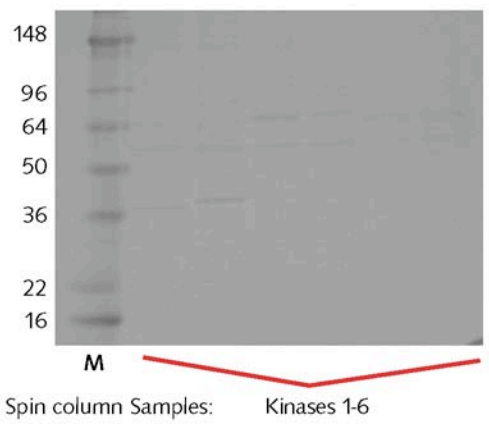

Figure 2: 10 µl elutions were run on a 4-20% Tris-glycine gel and stained with Coomassie Blue.

Results:

The His Spin columns demonstrate poor enrichment of each of the 6 kinases analyzed. Under these conditions, the technology did not reflect the expression status of these constructs and thus, did not provide an accurate prediction of large scale expression success.

Magnetic Bead-Based Technology

The bench-top, automated system, transfers magnetic Ni-charged particles through each of the purification phases for enrichment of histidine-tagged proteins.

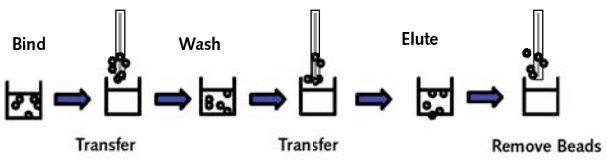

Figure 3: The Magnetic bead-based technology (MBBT) relies on the transfer of magnetic beads through binding, washing and elution phases. During incubation periods, the wells are shaken to aid the purification process.

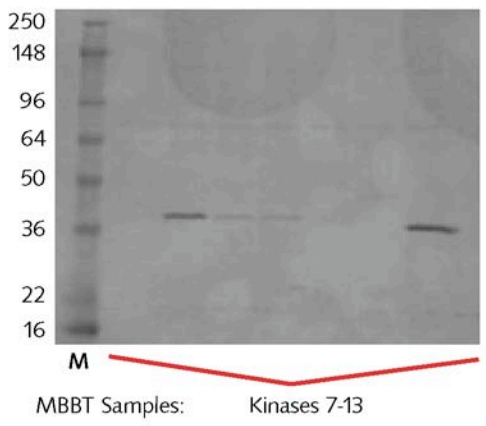

Figure 4: 10 µl elutions were run on a 4-20% Tris-glycine gel and stained with Coomassie Blue.

Results

The instrument demonstrates enrichment of 4 out of 7 kinases evaluated. Using the conditions described, the system did not provide enrichment of less well-expressed proteins, nor accurately illustrate differences in expression levels between those assessed.

PhyTip Column Technology

The PhyTip column technology (PhyNexus Inc.) utilizes small volumes of Ni resin encased in high-capacity micro-columns at the end of standard pipette tips. Researchers can rapidly enrich their target proteins from micro-volume samples using standard capture, wash and elution protocols, in a medium-throughput manner. Two platforms for this technology have been evaluated - the PhyNexus MEA automated system and the Caliper Sciclone ALH3000.

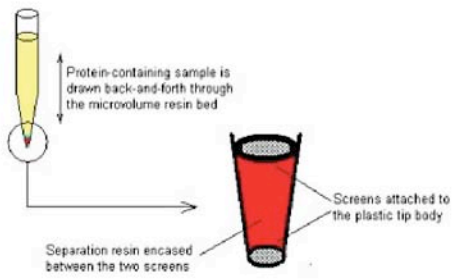

Figure 5. The PhyTip Column pipette tips.

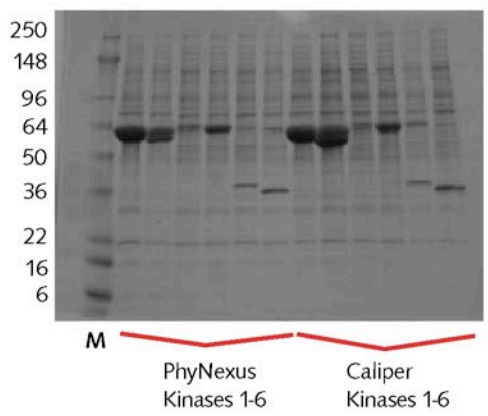

Figure 6: 10 µl elutions were run on a 4-20% Tris-glycine gel and stained with Coomassie Blue.

Results

Both the Caliper Sciclone ALH 3000 and the PhyNexus MEA Personal Purification System show comparable enrichment of all 6 kinases analyzed. In addition, the results accurately reflect the yields obtained in large-scale expression batches.

Summary Table

Factor	His Spin Columns	Magnetic Bead-Based Technology	PhyTip technology
Ease of use	Easy	Easy	Easy
Preparation time	5 min	15 min	30 min - Ultracentrifugation req'd
Time	10 min	15 min	14 min
Throughput	Manual - 24 samples	1-24 samples/run	1-12 samples/run
Walkaway	No	Yes	Yes
Cost/sample	£3.50	£5	£5 Phynexus MEA £3.15 Caliper
Scale Up	Poor Reflection	Poor Reflection	Accurate Reflection
Additional features	No other applications	DNA & RNA purification	Tips also available for a variety of common platforms - Tecan, Beckman, Caliper & Perkin Elmer

Conclusion

Here, we present the successful small-scale purification of baculovirus-expressed histidine-tagged proteins. For our application, with clarified insect cell lysates, the PhyTip technology consistently provides results on expression levels that are later mirrored in large-scale expression batches.