

# one nucleus insights



## Best Practices for Protein Purification and Advances in Affinity Chromatography

Prometic and One Nucleus hosted a 'Downstream Processing Innovation Day' that looked at the challenges faced by scientists working in the area of process development. More specifically highlighting current methods used for contaminant removal, the adoption of single use systems and comparisons of the best screening technologies. Here is a summary of the four presentations with slides attached that were showed on the day.

### ProMetic BioSciences; An overview

Since 1987 ProMetic BioSciences Ltd (PBL) has been pioneering design, development and manufacture of affinity purification technology for lab-scale and industrial-scale bioprocessing.

With over 25 years experience in the development of affinity products and design of new custom adsorbents, PBL is a world leader in its field. PBL offers an extensive range of off-the-shelf bioseparation products, for the recovery and purification of biologicals, as well as a variety of custom services including bespoke custom designed chromatography adsorbents, downstream process development and in silico modelling applications. PBL's product range covers a diverse array of applications from protein purification (such as; recombinant proteins, plasma proteins, antibodies, albumin fusion proteins and glycoproteins) to contaminant removal (such as; endotoxin, prions and serine proteases).

With an established manufacturing facility situated on the Isle of Man (UK), PBL can supply bulk adsorbent gel slurry for both developmental and regulated cGMP manufacturing applications in volumes sufficient to meet the requirements of the largest biomanufacturing processes. All of PBL's bioseparation products are supported by comprehensive regulatory support files and are produced in our controlled environment manufacturing facility under an ISO 9001 quality management system.

As well as our off the shelf product range, PBL also offers a range of custom services for downstream purification, to either identify/optimize a suitable existing PBL product, custom design a bespoke chromatography adsorbent specifically for your target biomolecule and application or develop and manufacture an adsorbent to your own specification.

To view the slides for PBL Corporate Overview Presentation (30-06-15) - Final, please click on the link below:  
<http://onenucleus.com/download.aspx?id=1599>

### High Throughput Screening Technologies

High throughput process development has become an essential element in the design and development of modern downstream processes. This technique shortens development time and increases the process knowledge available during the early phase of a development program.

ProMetic BioScience's (PBL) PuraPlate™ products are available pre-filled with PBL's commercially available range of adsorbents and provide a quick and effective platform for process optimization by enabling parallel screening of process conditions. Optimum conditions identified by PuraPlate™ can be applied to conventional chromatography columns for scale-up.

Each PuraPlate™ comprises 96 individual columns, each containing 0.25 mL of adsorbent and is operated under gravity with buffer/sample loading performed either manually using single or multi-channel pipettes or by automated liquid handling systems. The PuraPlate™ is easy to use operating under gravity without the need of either vacuum or centrifugation for sample collection. All buffer/sample loading can be performed either manually using single or multi-channel pipettes or by an automated liquid handling system. Buffer/sample applications are collected into 2 mL deep well collection plates which can then be analyzed using high throughput analytical techniques.

PuraPlate™ products can be utilized to generate a large volume of data quickly and effectively, whilst minimizing feedstock applications, for either process optimization (e.g. load, wash and elution strategies - varying pH, conductivity and additives) or adsorbent recycling and stability (e.g. Clean in Place (CIP) strategies - investigating numerous solvents, detergents and denaturants). Optimum conditions can then be scaled-up directly to conventional chromatography columns.

To view the slides for Chromatography Screening Technologies & Best Practices Presentation (30-06-15) - Final, please click on the link below:

<http://onucleus.com/download.aspx?id=1600>

## Disposable Chromatography Option

Increasing requirements for higher productivity and efficiency in the manufacture of biopharmaceuticals are providing the impetus for the adoption of single-use disposables in the bio-manufacturing. Over the last couple of years, interest has been growing in the use of pre-packed single use chromatography columns and in the use of devices with disposable fluid contact pathways. Recently, another option for the disposable single-use column has been introduced by ProMetic BioSciences. The Evolve™ range of columns (developed in conjunction with Delta Precision Ltd) have a disposable (and/or re-cycle able) process fluid contact pathway housed in a re-usable outer column. This concept is analogous to the accepted approach of a disposable plastic bag liner contained within a re-usable hard outer container.

The Evolve columns can be used in packed bed volumes ranging from 2L to 25L and retain the traditional axial column design familiar to most chromatographers. This versatile column range is applicable for use in research & development, process development and for small-scale clinical manufacturing. The Evolve columns are provided empty so the user can pack with their resin of choice. Column Refresh Kits are available, so the inner liner can be changed and the original column outer re-used.

Consequently, the Evolve columns provide a significant benefit (50% - 75% price reduction compared to the price of equivalent (unpacked) fixed-volume columns for multi-product facilities and who routinely perform short production campaigns at relatively small-scale.

To view the slides for Single Campaign Systems for DSP Presentation - Final, please click on the link below:

<http://onucleus.com/download.aspx?id=1601>

## Techniques for endotoxin removal

Endotoxin or lipopolysaccharides (LPS) are highly toxic components of the cell wall of Gram-negative bacteria & are often present in significant amounts in bacterial cell expression systems such as E. coli. The removal of endotoxin is extremely important for biotherapeutics, yet is difficult to remove. A number of downstream methods have been used including 'historic' affinity ligands such as polymixin B and L-histidine as well as anion exchange chromatography and membranes. These methods can work effectively but have distinct limitations including

toxicity, cost and non-selectivity. EtoxiClear™, One of ProMetic BioSciences proprietary synthetic chemical ligand adsorbents has the ability to remove endotoxin to 0.1EU/mg or less and has a binding capacity of >1,000,000EU/mL adsorbent. As EtoxiClear™ is not ion-exchange based, endotoxins can be removed from proteins with wide range of isoelectric points (pI 5 to 11) in buffers ranging from pH 4.0 to pH 8.0. EtoxiClear™ has been shown to provide >3.4log clearance of endotoxin from Fab fragments from an E. coli cell lysate, as well as from proteins such as IgG and HSA.

When testing processed samples using a LAL chromogenic kit, it is recommended to use Glucashield to remove β-D-Glucan interference which may artificially increase the signal. EtoxiClear™ is fully scalable and is shown to remove endotoxins from ~40EU/mg down to <0.1EU/mg (~3 log clearance) at a 5mL pre-packed bench scale column as well as when packed in an 780mL Evolve™ column. EtoxiClear™ can also remove endotoxin from a range of buffer solutions including PBS and other balanced salt solutions used in cell culture.

In summary, there are several techniques available for the removal of endotoxin, but none are as selective, cost-effective, non-toxic and with the performance that EtoxiClear™ can provide.

To view the slides for Techniques for endotoxin removal Presentation (30-06-15) - Final, please click on the link below:

<http://onucleus.com/download.aspx?id=1602>