INSECT CELL/BACULOVIRUS PRODUCTION

PEF #	GENE NAME	TRANSFER VECTOR	BEVS	MOLECULAR WEIGHT
2015-XXXX	XXXX	pBAC1	*flash*BACULTRA^{TM}	36.0 kDa

EXPRESSION METHOD OVERVIEW:

Insect cells *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (High Five^{TM}) are routinely grown and maintained in ESF 921 insect cell media.

VIRUS AMPLIFICATION

Amplification of the Passage 3 recombinant baculovirus (P3 BV) was conducted in shake flask format. Sf9 cells were seeded at 1 x 10^6 cells/mL. An appropriate volume of the recombinant Passage 2 virus seed stock was used as inoculum. The cell density and viability were monitored and the culture supernatant was harvested (P3 BV) when the cells appeared well infected with the virus.

EXPRESSION

Expression culture was set up using the infection parameters below. Briefly, 1 L High Five^{TM} cells were seeded at mid-log phase in a 2.8 L fernbach flask and infected with an appropriate volume of P3 BV. The cell density and viability were monitored and the expression culture was harvested at 72 hours post infection (hpi) by centrifugation at 12,000 *g* for 20 min.

Table 1 | Expression Parameters

Total Culture Volume	1 L
Cell Line	High Five^{TM}
Cell Concentration at Infection	1.5 x 10^6 cells/mL
Multiplicity of Infection	5 (estimated)
Media	ESF 921
Temperature	27˚C
Speed	120 rpm
Time of Harvest	72 hpi

PRIMARY PURIFICATION METHOD OVERVIEW:

CROSSFLOW CONCENTRATION

Supernatant from 1 L expression culture was passed through a 0.45 μm filter and concentrated to 180 mL using a 10 kDa nominal molecular weight cut off Hydrosart ultrafiltration cassette (Sartorius Crossflow Systems).

IMMOBILISED METAL AFFINITY CHROMATOGRAPHY (IMAC)

Concentrated supernatant was filtered before loading onto an IMAC column pre-equilibrated in wash buffer. Unbound protein was removed with 20 column volumes (CV) of wash buffer. Elution was performed using a step to 4% elution buffer (B) for 5 CV, followed by a gradient from 4 – 100% B over 15 CV and holding at 100% B for a further 10 CV, collecting 2 mL fractions. All steps were performed on an AKTAxpress FPLC system (GE Healthcare) at a flow rate of 5 mL/min, 4˚C (Figures 1 and 2).

ANALYSIS

Purification samples were loaded onto a 4-12% Bis-Tris SDS-PAGE gel and run under denatured and reduced conditions (Figure 3). Analysis was performed using a Bio-Rad Chemi-Doc™ XRS+ imaging system. The molecular weight was calculated using ProtParam (http://web.expasy.org/protparam/).

Table 2│ IMAC Purification Details

Column	1 mL Ni Sepharose Excel (GE Healthcare)
Wash Buffer	20 mM Sodium Phosphate, 500 mM NaCl, 20 mM Imidazole, pH 7.7 (4˚C)
Elution Buffer	20 mM Sodium Phosphate, 500 mM NaCl, 500 mM Imidazole, pH 7.7 (4˚C)

RESULTS:

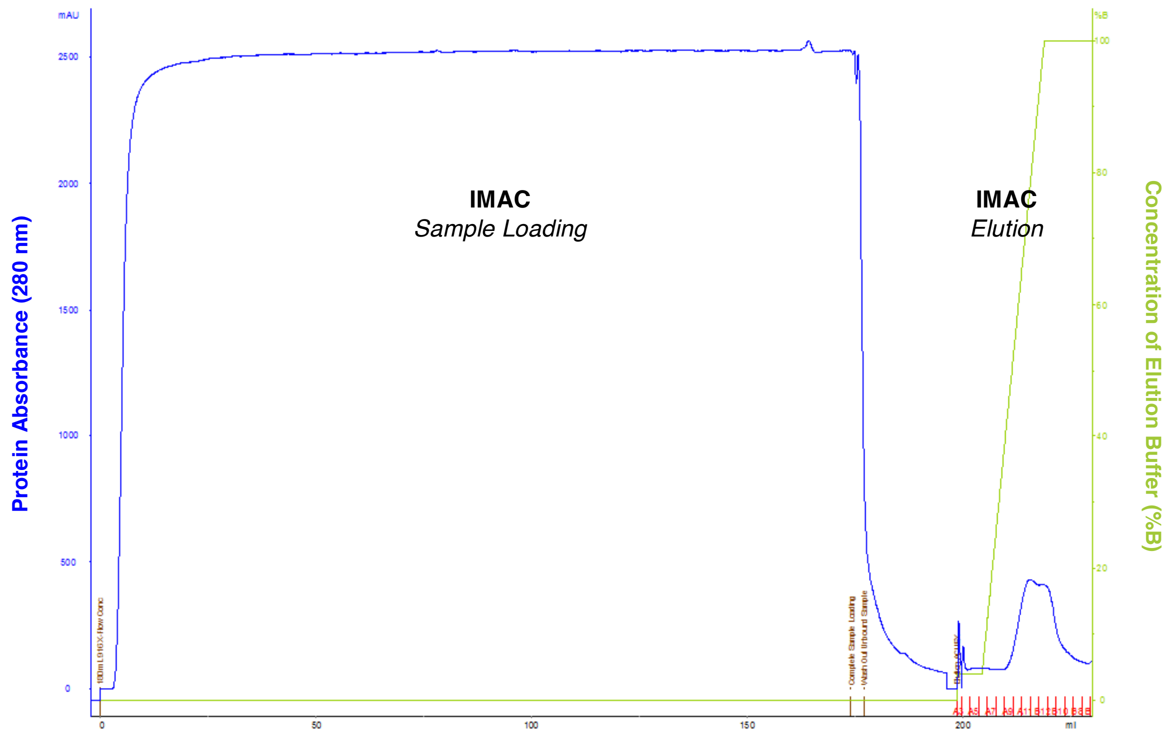

Figure 1│ **2015-XXXX IMAC purification chromatogram**

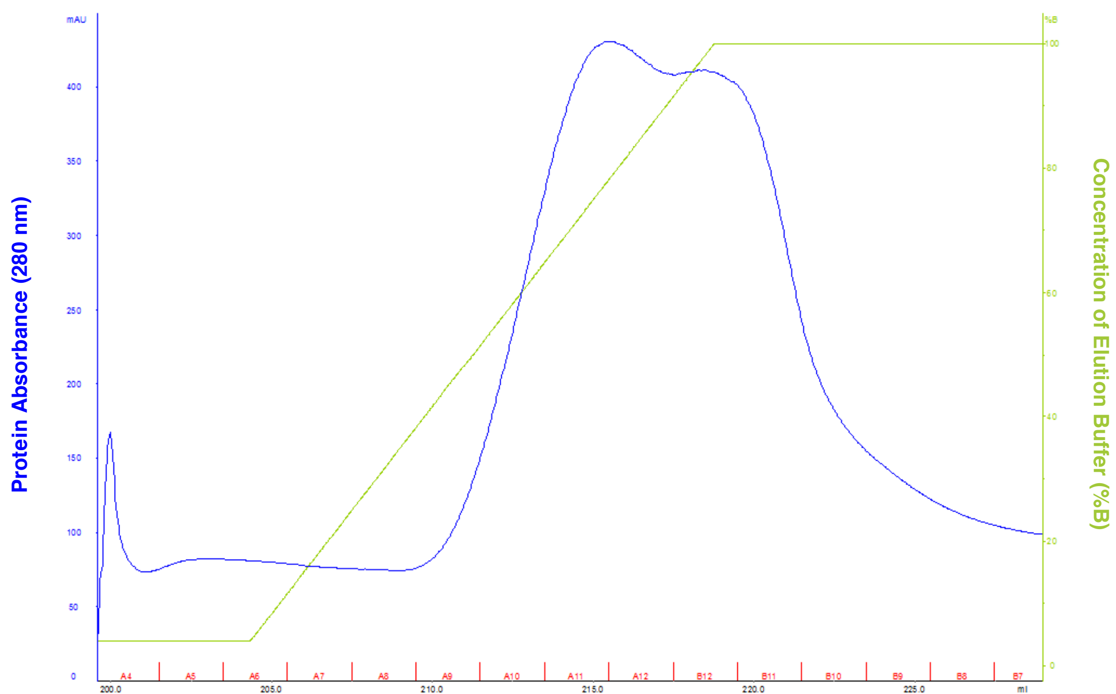

Figure 2│ **2015-XXXX IMAC purification chromatogram (elution)**

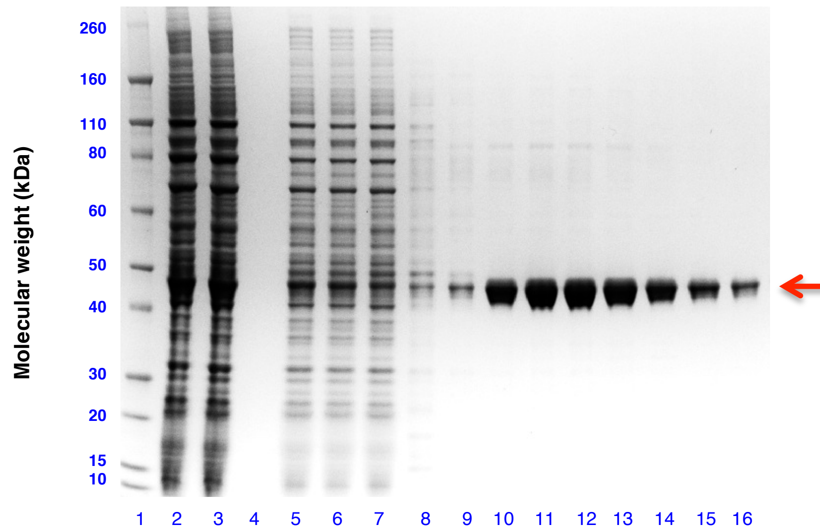

Figure 3 | 2015-XXXX IMAC purification analysed by SDS-PAGE

Lane	Sample	Lane	Sample
1	Novex® Sharp Pre-stained MW ladder	9	IMAC Elution Fraction 1
2	Culture Supernatant*	10	IMAC Elution Fraction 2
3	Filtered Supernatant*	11	IMAC Elution Fraction 3
4	Crossflow Permeate (Waste)*	12	IMAC Elution Fraction 4
5	Crossflow Filtrate (Concentrated)	13	IMAC Elution Fraction 5
6	IMAC Load (Filtered)	14	IMAC Elution Fraction 6
7	IMAC Flow Through	15	IMAC Elution Fraction 7
8	IMAC Wash	16	IMAC Elution Fraction 8

*Samples TCA precipitated (concentrated 15x)

SECONDARY PURIFICATION AND PROTEIN STORAGE METHOD OVERVIEW:

BUFFER STABILITY SCOUTING

A small volume of IMAC purified 2015-XXXX was buffer exchanged into phosphate buffered saline (PBS) using a PD SpinTrap G-25 column (GE Healthcare). An aliquot of this sample was snap-frozen in liquid nitrogen and stored at -80˚C overnight, the remainder stored at 4˚C, to determine a suitable storage condition. Analysis indicated that 2015-XXXX was stable in PBS, and that no significant loss of protein occurred following storage at both 4˚C and -80˚C in the absence of any additives (results not shown).

SIZE EXCLUSION CHROMATOGRAPHY (SEC)

IMAC elution fractions corresponding to 2015-XXXX were pooled and concentrated using an Amicon Ultra-15 centrifugal filtration device with a 10 kDa molecular weight cut off (Millipore). The concentrated sample was filtered and loaded onto an SEC column pre-equilibrated in storage buffer, collecting 2 mL fractions. All steps were performed using an AKTAxpress FPLC at a flow rate of 2 mL/min, 4˚C. Bovine serum albumin (BSA) and blue dextran standards were also run under the same conditions to enable an estimation of protein molecular weight (Figure 4). Samples from the purification process were analysed by SDS-PAGE (Figure 5).

Table 3 | SEC Purification Details

Column	HiLoad 26/600 Superdex 200 (GE Healthcare)
Strorage Buffer	Phosphate buffered saline (PBS)

PROTEIN CONCENTRATION AND STORAGE

SEC fractions corresponding to 2015-XXXX were pooled and passed through a 0.2 µm filter. The filtered protein was snap-frozen in liquid nitrogen in 1.6 mL aliquots and stored at -80˚C.

ENDOTOXIN MEASUREMENT

An endotoxin test was carried out on the final sample using an Endosafe-PTS Limulus Amebocyte Lysate (LAL) test kit (Charles River Laboratory).

RESULTS:

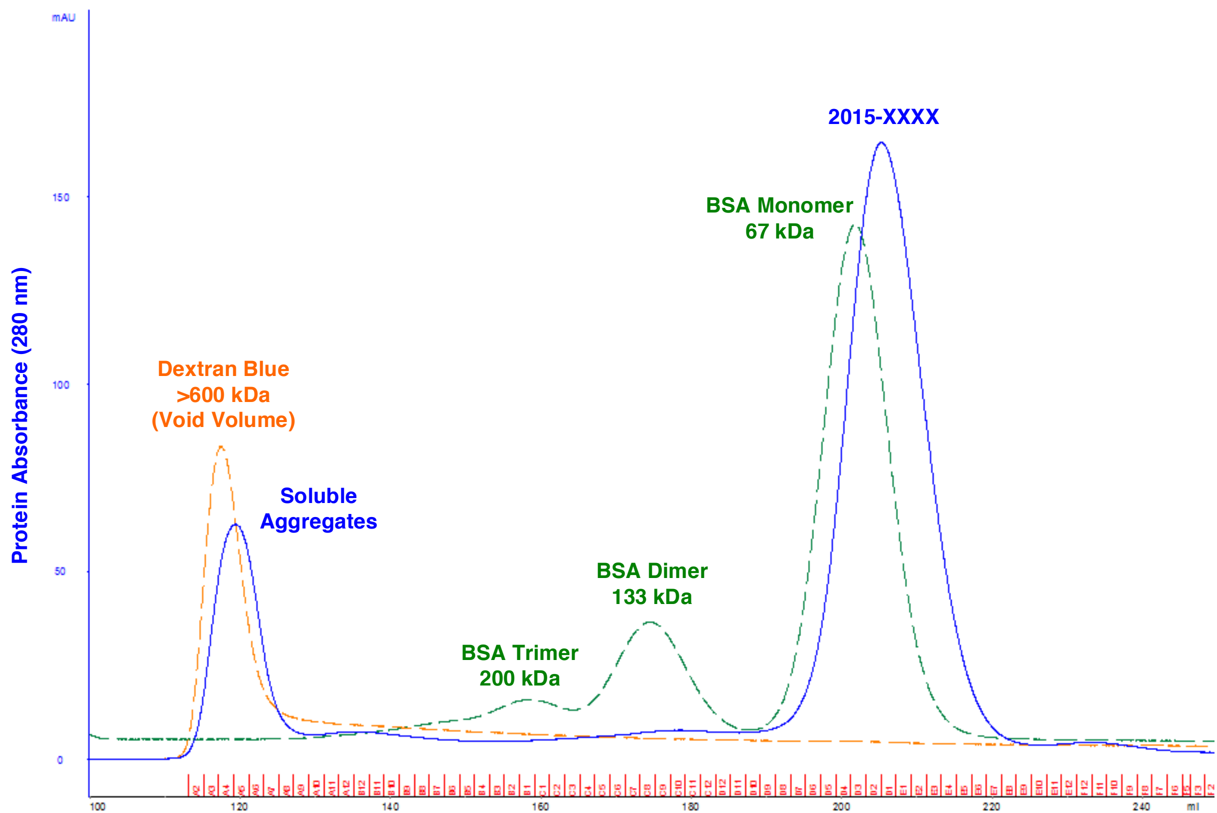

Figure 4 | 2015-XXXX SEC purification chromatogram. Overlayed with BSA (green) and blue dextran (orange) standards.

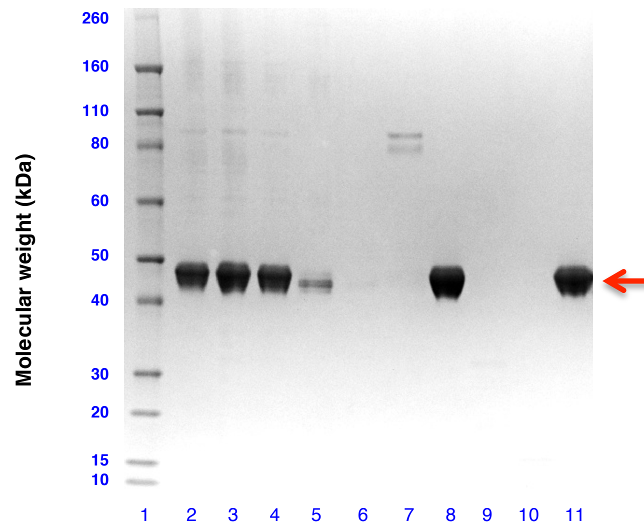

Figure 5 | 2015-XXXX SEC purification analysed by SDS-PAGE

Lane	Sample
1	Novex® Sharp Pre-stained MW ladder
2	IMAC Pooled Elution Fractions
3	Concentrated Elution Fractions*
4	SEC Load (Filtered)*
5	SEC Elution Fraction 1
6	SEC Elution Fraction 2
7	SEC Elution Fraction 3
8	SEC Elution Fraction 4
9	SEC Elution Fraction 5
10	SEC Elution Fraction 6
11	SEC Pooled Fractions (Final Sample)

*Samples diluted 1:2

Sample Information:

Table 4 | Purified Protein Information

Final Protein	2015-XXXX
Volume	32 mL
Protein Purity	>99%
Target Protein Concentration	260 μg/mL
Target Protein Yield	8.32 mg
Endotoxin Level	<0.5 EU/mL <1.92 EU/mg
Buffer	Phosphate Buffered Saline (PBS)
Storage Conditions	-80˚C, 20 x 1.6 mL aliquots

Comments:

- Target protein concentration was estimated by absorbance at 280 nm using a NanoDrop spectrophotometer, taking into consideration the protein's extinction coefficient and sample purity. Target protein purity was estimated by band intensity analysis using ImageLab software.

Research groups using the UQ Protein Expression Facility (PEF) must acknowledge the contribution provided by the Facility. Please acknowledge UQ PEF's provision of Services in your written publications and/or oral presentations relating to the Processed Material, thank you.