BEVS Insect Cell Protein Production: Novel High Volume Shake Flask Expression

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Abstract

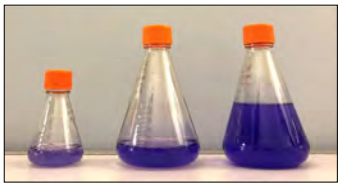
The production of recombinant proteins in the baculovirus expression vector system (BEVS) in modern R&D laboratories utilises either shake flasks or cell culture bags for insect cell culture scale-up. Typical insect cell culture in Erlenmeyer flasks utilise a limited cell culture volume, typically 30% maximum, ostensibly to ensure adequate culture aeration. A typical volume for insect cell scale-up is 1L in a 3L Erlenmeyer flask. To scale up past the 1L volume, multiples of such cultures are often combined or, more commonly, expression is carried out in a cellbag-based system, such as the Wave BioReactor, where volumes of 5L, 10L or 25L are common. Cell culture in a cellbag system often requires investment in rocker platforms, air pumps, media pumps and tube welders to facilitate the sterile media transfers outside of a biosafety cabinet. However, in this study, it was found that, depending on flask type, insect cell cultures demonstrate standard growth curves when the culture volume represents as much as 80-90% of the stated flask volume. In addition, under these conditions, recombinant protein expression levels in BEVS are similar to those carried out in cellbags. These 'high volume' shake flask cultures thus allow for the elimination of cellbag technology for scale-up protein production, with substantial time and cost savings.

Experimental Procedures

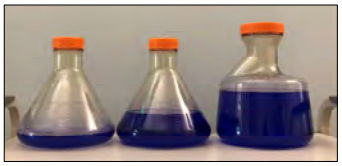
A. Insect cell growth profiles

*Sf*9, *Sf*21 and High Five (BTI-TN-5B1-4) insect cells were seeded at ~8x10^5 cells/mL in a variety of non-baffled Erlenmeyer flask sizes from Corning and using various volumes of ESF-921 medium. Cultures were grown in either a Climo-Shaker ISF4-X or a ISF-4-W Kuhner shaker at 27°C, each with a 25mm throw. The culture details were as follows:

Corning Cat#	Flask Volume (L)	Culture Volume (L)	RPM	Graph Symbol
431144	0.25	0.1	130	
431147	1	0.3	130	
431147	1	0.8	130	
431252	3	1	90	
431252	3	2.5	130	
431685	5	4.5	130	



Corning 0.25L Erlenmeyer Flask #431144 0.1L fluid | Corning 1L Erlenmeyer Flask #431147 0.3L fluid | Corning 1L Erlenmeyer Flask #431147 0.8L fluid



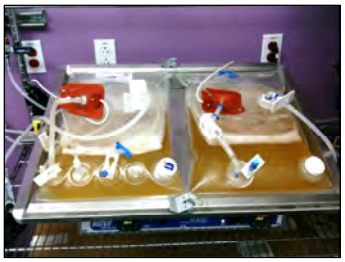
Corning 3L Erlenmeyer Flask #431252 1L fluid | Corning 3L Erlenmeyer Flask #431252 2.5L fluid | Corning 5L Erlenmeyer Flask #431685 4.5L fluid

Corning flask sizes and culture volumes compared in this study

B. Recombinant protein expression: flasks vs cellbags

Four protein kinases were selected as test proteins to compare protein production levels in baculovirus-infected *Sf*21 insect cells in high-volume shake flask cultures versus Wave cellbag cultures. The test proteins included full-length PRKD1 (104 kDa) and the kinase domains, all 37 kDa, of HPK1, MST2 and MST3. All protein ORFs were cloned into an in-house transfer vector that appended the N-terminus with a polyhistidine purification tag. Recombinant baculoviruses were generated by using the Bac-to-Bac method. Scale-up protein expressions were carried out in *Sf*21 insect cells (~2x10^6 cells/mL) at an moi ~1 for 72h at 27°C in either 4.5L volumes in 5L Corning flasks (part # 431685) at 130 rpm in a Kuhner Climo-Shaker ISF4-X, or in 5L volumes in 10L Wave cellbags (part number CB0010L10-34 Rev AG) and using Wave BioReactors (model System 20/50EHT). The same cell stock was used to seed both the flask and the Wave cellbag for each parallel expression. Cell samples (3x 50mL) were harvested at 72h post-infection from each expression and were independently purified by cell lysis and IMAC (immobilized metal affinity chromatography). The eluate samples were analysed by protein assay (Bradford method) and by SDS-PAGE.

Corning 5L flasks containing 4.5L cell culture



Wave10L cellbags containing 5L cell culture

Results

A. Insect cell culture

Fig. 1. Effects of culture volume and flask size on *Sf*9 insect cell growth.

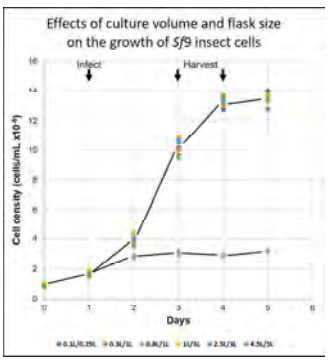


Fig. 2. Effects of culture volume and flask size on *Sf*21 insect cell growth.

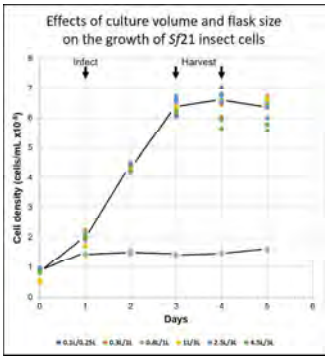
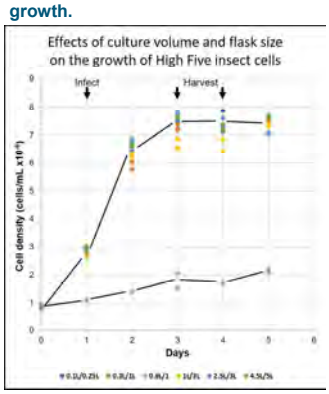


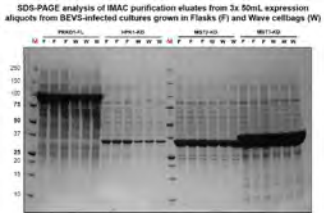
Fig. 3. Effects of culture volume and flask size on High Five insect cell growth.



B. Recombinant protein expression & purification

Scale-up protein expressions were carried out in either 4.5L volumes in 5L Corning flasks or in 5L volumes in 10L Wave cellbags using Wave BioReactors as described in Experimental Procedures. After 72h, 3x 50mL samples from each expression were independently purified and the IMAC eluates were analysed by protein assay by SDS-PAGE.

Fig. 4. Purification of four kinases expressed at 4.5L in 5L Flasks (F) or at 5L in 10L Wave cellbags (W)



The three independently purified IMAC eluates for each expression were combined and each Flask and Wave cellbag sample compared side-by-side by SDS-PAGE.

Kuhner Climo-Shaker ISF4-X with a mixture of 2.5L and 4.5L insect cell expressions in Corning 3L and 5L flasks, respectively.

Fig. 5. Side-by-side comparison of four proteins expressed at 4.5L in 5L Flasks (F) or at 5L in 10L Wave cellbags (W)

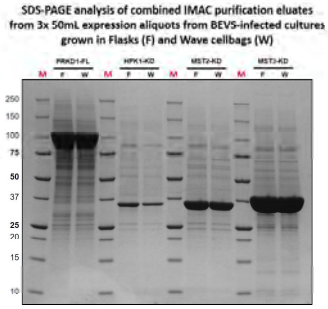
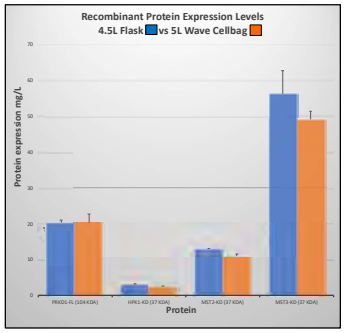


Table 2 & Fig. 6. Protein concentrations in the IMAC eluates (from three separate parallel purifications of four proteins)

Protein	Corning Flask mg/L	Wave cellbag mg/L
PRKD1-FL (104 kDa)	20.3 ± 0.7	20.3 ± 2.4
HPK1-KD (37 kDa)	3.1 ± 0.1	2.5 ± 0.1
MST2-KD (37 kDa)	13.0 ± 0.1	11.0 ± 0.6
MST3-KD (37 kDa)	56.4 ± 6.4	49.2 ± 4.0



Discussion & conclusions

The baculovirus expression vector system (BEVS) has emerged as the primary R&D platform for the production of recombinant eukaryotic proteins. Expression cultures have traditionally been of two options: A) a 1L culture or multiple 1L cultures in 3L shake flasks, or B) conduct protein expression in cellbags on rocking platforms. Wave Bioreactor rockers have a capacity for 2x 5L or 1x 10L on the small platform, or 2x 10L or 1x 25L on the larger platform. Each pair of 5L or 10L cultures requires a rocking platform. In addition, all cellbag cultures require an air pump and an exhaust heater for each separate bag, and the larger volume bags require additional equipment including tube welders and pumps to transfer media outside of a biosafety cabinet. In contrast, by using the high-volume 5L Corning shake flask described here, a single Kuhner Climo-Shaker ISF4-X can accommodate 6x 4.5L cultures per platform that, with three platforms, equates to 18x 4.5L cultures in total. Thus, a single shaker may be used for 1x 81L, 2x 40.5L, 4x 18L + 1x9L, 9x 9L or 18x 4.5L expressions, or various combinations thereof. The cost of a 5L Corning flask is approx. one-fifth the cost of a 10L (5L working volume) Wave cellbag, although there are less expensive cellbag alternatives to Wave. Nevertheless, a substantial benefit is realised from the ease of set-up of high-volume shake flasks versus the rather cumbersome cellbag set-up, in addition to the fact that flasks may be set up in the biosafety hood and do not require time consuming media transfers outside the biosafety cabinet. High-volume shake flask expression represents an excellent alternative to cellbags.