

# Swellscreen- Rapid Baculovirus Titration Method in Microplate format

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## Objective

The aim of this study was to develop a high-throughput swell-based method for baculovirus quantification by down-scaling of an existing method.

## Introduction

Traditionally, baculovirus titers have been determined with plaque assays and end-point dilution<sup>1</sup> whilst more recent titration methods are based on e.g. immunology, flow cytometry and quantitative real-time PCR<sup>2,3,4</sup>. However, each method has its drawbacks, such as being time-consuming or difficult result interpretation. In contrast, swell-based assays, where cell swelling after infection is measured and correlated to the virus titer, have shown to be rapid and simple<sup>5,6</sup>. We have previously used a swell-method described by Liu and Hong (2004) for baculovirus titration, and the CEDEX cell analysis system (Innovatis) for cell diameter measurement. To increase throughput and decrease CEDEX workload, we have developed a scale-down protocol in microplate format. Further, the new method was validated against BacPAK (Clontech), a well-known immunological assay.

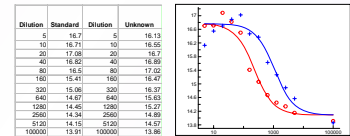
## Experimental set-up



Sf21 cells were seeded into a 96-well microplate, infected with serial dilutions of baculoviruses (one virus/row A-G), and the plate incubated at 27°C



After 24 h, the cell diameter average in each well was determined with CellScreen automated image-based screening system (Innovatis).



Dose response curves resulting from infections of viruses with known and unknown titres, respectively, were compared, and the titres of the unknown viruses calculated from the midpoint shift.

## Scale-down results

The titres measured with the Swellscreen microplate method correlated very well with the titres from the original CEDEX method (fig. 1A), and the scale-down approach was thereby considered to be valid.

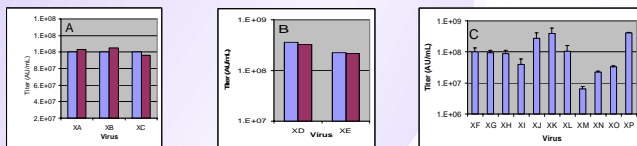


Figure 1. A) Comparison of Swellscreen titers (blue) with titers from the original method (purple). B) Titration of undiluted (blue) and 2x diluted (purple) viruses. The dilution factor is compensated for in the figure. C) Titration of 11 different viruses at 3 (XF-XL) or 2 (XM-XP) different occasions.

The Swellscreen linearity was examined by measurement of diluted and undiluted viruses. After compensating for the dilution factor (2x) the titres were similar (fig. 1B), which implies that the method is linear within the measured range. Further, the variability between different positions within one microplate was investigated by analysing one virus in hexaplicate (rows B-H) in the same plate. After 24h the average cell diameter was measured, and the difference between corresponding wells calculated. The results showed a standard deviation of only 0.7%, as relative to the measured diameter value (not shown), which demonstrates that the assay is position independent.

The consistency of Swellscreen was determined by repeated titration of 11 different viruses, with either intra- or extracellular recombinant products (fig. 1C). The resulting errors were sufficiently small to make Swellscreen a useful baculovirus titration method for our purposes.

## Validation with BacPAK

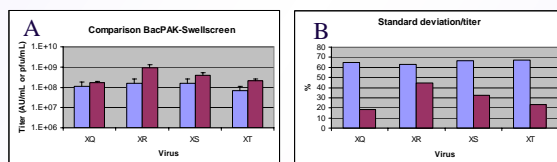


Figure 2. A) Comparison of Swellscreen titers (purple) with BacPAK titers (blue). B) Relative errors (standard deviation/titer) of BacPAK (blue) and Swellscreen (purple), respectively.

The Swellscreen titers were compared with titers measured with the commonly used BacPAK immunoassay (Clontech). BacPAK is based on antibodies against the baculoviral coat protein gp64, which is displayed on the surface of infected cells. BacPAK average titers were in general lower than Swellscreen titers (fig. 2A), but the order highest titer- lowest titer was similar. When comparing the standard deviation, Swellscreen data proved to be the more reliable compared to BacPAK data (fig. 2B).

## Discussion

The Swellscreen scale-down approach with CellScreen (Swellscreen) generated titers comparable with the original CEDEX method. Further, Swellscreen engendered reasonably consistent results, and was subjected to the least relative errors in comparison with BacPAK immunoassay. However, discrepancies existed in titer comparisons with BacPAK. The highest titer lowest titer order was similar for both assays, but Swellscreen titers were in general higher and the differences exaggerated at higher virus concentrations. With hindsight dissimilarities may have been expected since the two methods do not measure the same parameters, but the fact that the differences were not constant is intriguing. Two swell-based methods for baculovirus titration have been described<sup>5,6</sup>. However, none of these methods is thoroughly validated against other assays and the titer interpretation may benefit from further discussion.

Still, swell-based assays gives a good picture of the physiological response resulting from infection of an unknown virus stock, and easily enable discrimination between a functional and a defective virus. When using Swellscreen, multiple viruses may be analysed simultaneously, and together with its simplicity, speed and low cost, it is well suited for virus quality check before recombinant protein production.

## Conclusions

- The scale-down approach was successful
- Swellscreen generated the least relative errors in a comparison with BacPAK immunoassay
- Swellscreen titers may not be directly correlated to BacPAK titers
- Swellscreen is a high-throughput, low-cost and rapid alternative for quality verification of baculoviruses

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## References

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