



Comparison with Plaque Assay for Baculovirus Quantitation

In a blinded study, InDevR’s ViroCyt Virus Counter was compared to traditional viral plaque assay for the quantification of a baculovirus stock. For a series of dilutions prepared from the baculovirus stock, the Virus Counter measurement made by InDevR was linearly correlated to the plaque assay measurements conducted by both Protein Sciences Corporation and Baylor College of Medicine. The ViroCyt Virus Counter provides higher accuracy and a greatly reduced time to result compared to viral plaque assay.

PLAQUE ASSAY METHODS FOR VIRUS QUANTITATION

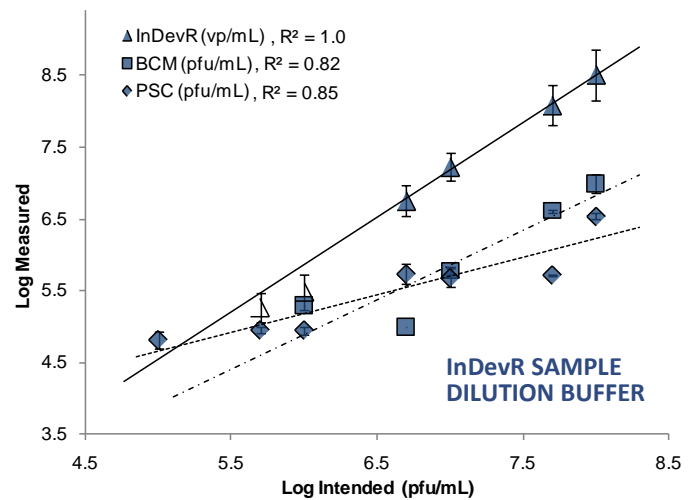
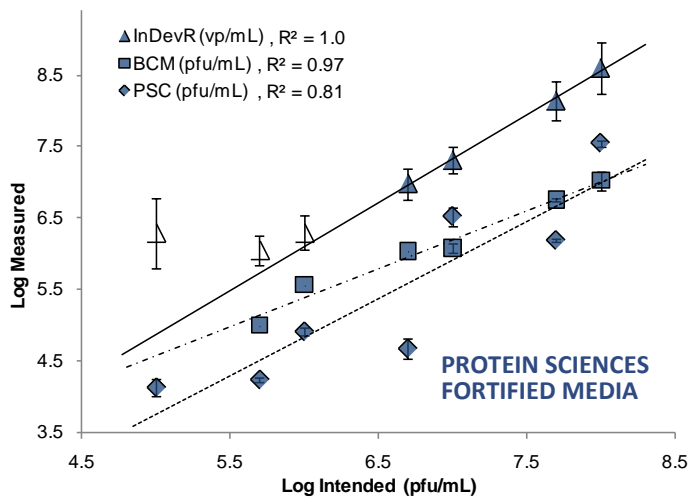
- Stocks prepared by Protein Sciences Corporation with initial plaque assay titer of **1.7 x 10⁸ pfu/mL**
- Dilution series prepared in two diluents (Protein Sciences Fortified Media and InDevR Sample Dilution Buffer), and provided in blinded fashion to Baylor and InDevR (shipped on ice, stored at 4°C until analysis)

Protein Sciences (PSC) Method	Baylor College of Medicine (BCM) Method
Each dilution tested in triplicate	Each dilution tested in triplicate
Sf-9 cells grown in TNM-FH to ~1 x 10 ⁶ cells/mL	Low passage Sf-9 cells grown in Grace’s insect media plus 0.1% Pluronic F68
2.5 x 10 ⁶ cells added to petri dish and incubated 60 min at room temp.	~1.25 x 10 ⁶ cells plus 10% heat inactivated FBS added to petri dish, incubated 60 min at 27°C
Media then removed, 1mL virus sample added, incubated at room temp. for 60 min w/manual rocking	Media then removed, 1mL virus sample added, incubated at 27°C for 60 min with automated rocking
50% mix of 2x TNM-FH/agarose overlay added and allowed to rest 45 min	Grace’s/agarose overlay added, vented 5 min, wrapped individually in parafilm
Dishes incubated at 27C for 6 days, and plaques subsequently counted under low magnification	Dishes incubated at 27°C for 7 days, plaques subsequently stained blue with 0.4% trypan blue, counted using inverted microscope

VIRUS COUNTER METHOD

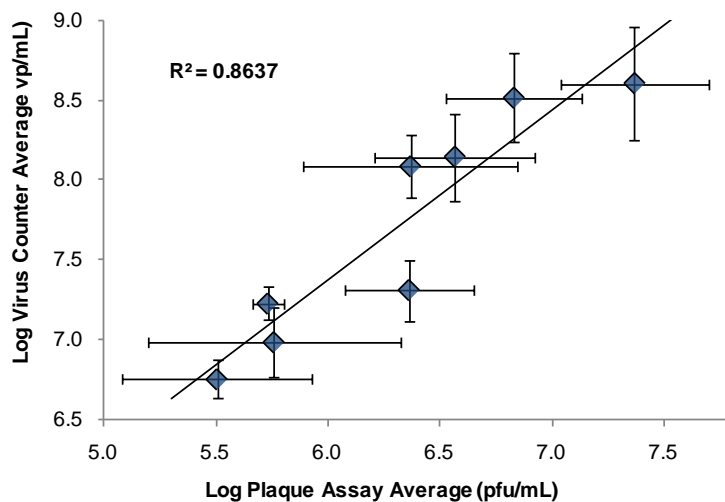
- Instrument validated daily with non-biological positive control to ensure proper function/performance
- Virus samples diluted 10x in Sample Dilution Buffer; Combo Dye solution then added to 200 µL of diluted sample and incubated at room temperature for 30 min
- All samples analyzed at least 5 times over multiple days (by multiple users and on multiple instruments)
- Each sample required ~10 min analysis time

GRAPHICAL COMPARISON OF RESULTS



- Virus Counter results (vp/mL) are typically higher than plaque assay results (pfu/mL), as all intact particles are counted, regardless of ability to infect cells
- All 3 methods show expected linear response with dilution and a statistically significant Pearson correlation with intended titer ($p < 0.01$ for all 3 methods)

VIRUS COUNTER RESULTS ARE STRONGLY CORRELATED TO TRADITIONAL PLAQUE ASSAY RESULTS



IMPROVED PRECISION

- Inter-lab variation observed for plaque assays
- Calculated standard errors of the mean :

Virus Counter: 0.09 ± 0.03 vp/mL

Plaque assay: 0.14 ± 0.06 pfu/mL

FASTER TIME TO RESULT

- Virus Counter analysis time is less than 10 minutes per sample
- All Virus Counter analyses (**124 independent measurements**) completed in under 3 days
- Plaque assay results by both institutions had a time to result of at least 2 weeks

Adapted from:

Ferris, M.M., Stepp, P.C., Ranno, K.A., Mahmoud, W., Ibbitson, E. Jarvis, J. Cox, M.M.J. Christensen, K., Votaw, H. Edwards, D.P., Rowlen, K.L. (2011) **Evaluation of the Virus Counter[®] for rapid baculovirus quantitation**, *Journal of Virological Methods*, 171(1), 111-116.