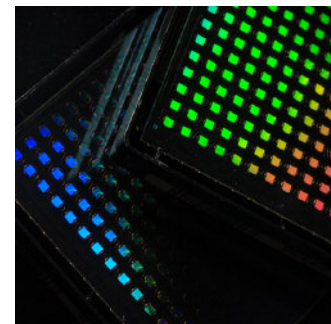


# Corning® Epic® System: Real-time Monitoring of Cell Response to Virus Infection



## SnAPPShots

A brief technical report  
from the Corning  
Applications Group

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## Introduction

Traditional cell-based assays for monitoring viral infection utilize either a cytopathic effect (CPE) assay, a plaque assay, a PCR amplification of viral genetic materials, a gene reporter assay, or a combination of the above. These low-throughput assays are time consuming as they involve multiple steps from cell seeding to RNA or DNA extraction from infected cells. For example, the most commonly used CPE assay involves cell seeding, virus infection, microplate washing, cell staining, lysis and final read. The whole process can take more than 72 hours from beginning to end. A PCR-based or gene reporter-based assay requires careful handling of cellular materials, or needs intense genetic manipulation of cell lines used. In this Application Note, we report the use of the Epic System, an optical sensor-based label-free HTS platform (1), to monitor real-time the HeLa cell response to human rhinovirus (HRV) infection. It is shown that HeLa cells respond to HRV in a time-dependent and virus dose-dependent manner. Furthermore, we demonstrate that the Corning Epic System can detect 100-fold fewer viral particles infecting target cells in as few as 10 hours.

## Materials and Methods

### Cell Culture

HeLa cells were cultured in DMEM supplemented with 10% fetal calf serum at 37°C with 5% CO<sub>2</sub>. Cells used were between passages 5 and 20. Cells were seeded in a Corning cell-based uncoated Epic microplate (Corning Cat. No. 5040) at an optimal seeding density of 15,000 cells/well, and grown overnight before use in the virus infection assay.

### HRV14 and HRV16 Preparation

Two different HRV serotypes, HRV14 and HRV16, were used. The viruses were purified according to Rossmann and co-workers (2). Briefly, HeLa cells were grown to confluency and infected with HRV at a multiplicity of infection (MOI) of 5 pfu/cell. Cells were harvested, homogenized and centrifuged. The virus was precipitated from cell lysate, treated with DNase and RNase, and ultra-centrifuged in a 0 to 40% sucrose gradient. Virus bands were collected and quantified with a spectrophotometer using an extinction coefficient of 7.7 at 260 nm.

### PFU Determination

The PFU assay was performed according to Rueckert et al. (3). HeLa cells were seeded in a 12 well microplate at 50% confluency in culture media. The virus was added to each well the following day, incubated at room temperature, washed, and covered with low melting point agarose (Invitrogen™). The microplate was incubated at 35°C for 60 to 72 hours. The plaques were observed by staining the monolayer with a 0.25% solution of crystal violet in 20% methanol.

### CPE Assay

HeLa cells were seeded at 50% confluency in a 96 well microplate. On the day of the assay, culture medium was removed from the wells, and 100 µL of diluted virus was added and incubated at 35°C for the time specified. At the time points indicated, the cells were stained with 0.25% crystal violet prepared in 20% methanol, washed extensively with water, and lysed with 1% SDS. Absorbance at 570 nm was then measured.

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## Corning® Epic® Assay

HeLa cells were seeded in an uncoated Corning cell-based Epic microplate at a density of 15,000 cells/well. The microplate was incubated in a 37°C incubator with 5% CO<sub>2</sub> overnight. The following day, the virus stock was diluted with culture medium to the desired concentrations and added to the microplate with a 384 tip liquid pipettor. The microplate was immediately read to record the early phase response, and the late phase response was read 2 hours later. Data was recorded as a function of time and plotted as time traces.

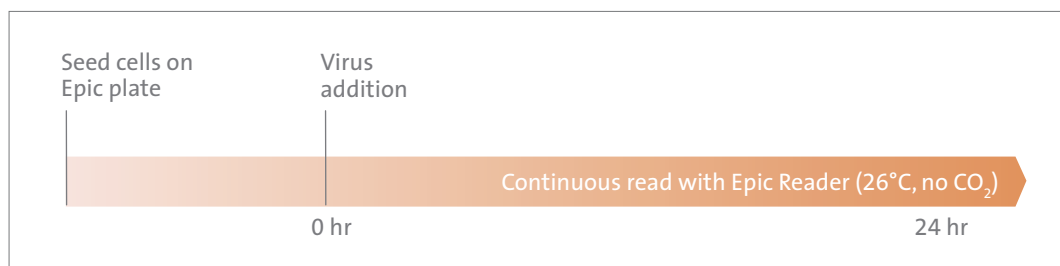
## Results

Figure 1 is a schematic of the Corning Epic assay. The Epic time trace represents the entire viral life cycle, starting with virus attachment and entry into cells, and ending with viral release from the cells (Figure 1). The time trace is characterized by an early response phase which is virus dose dependent, a middle lag phase, and a late response phase represented by a large decrease in the Epic signal.  $T_m$ ,

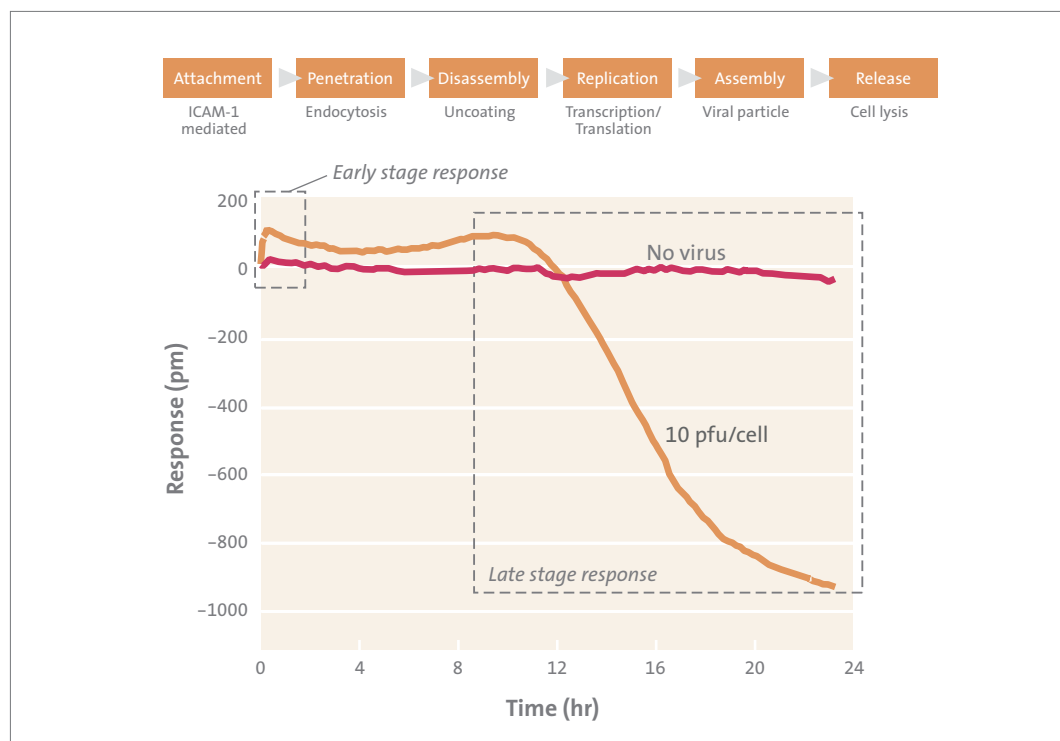
defined as the time at which half of the maximal late phase signal is achieved, shifts to the left with increasing virus dosages (Figures 2 and 4). The nature of the early response is not clear, but the late phase response is likely due to cell death, lysis and/or detachment resulting from virus infection.

The Epic signal increased rapidly in the first 20 minutes upon virus addition, and decreased gradually in the next 2 hours (Figure 3A). The magnitude of the signal was virus dose-dependent with the highest signal observed with the highest virus dose (Figure 3B). Similar response to HRV16 was observed (data not shown).

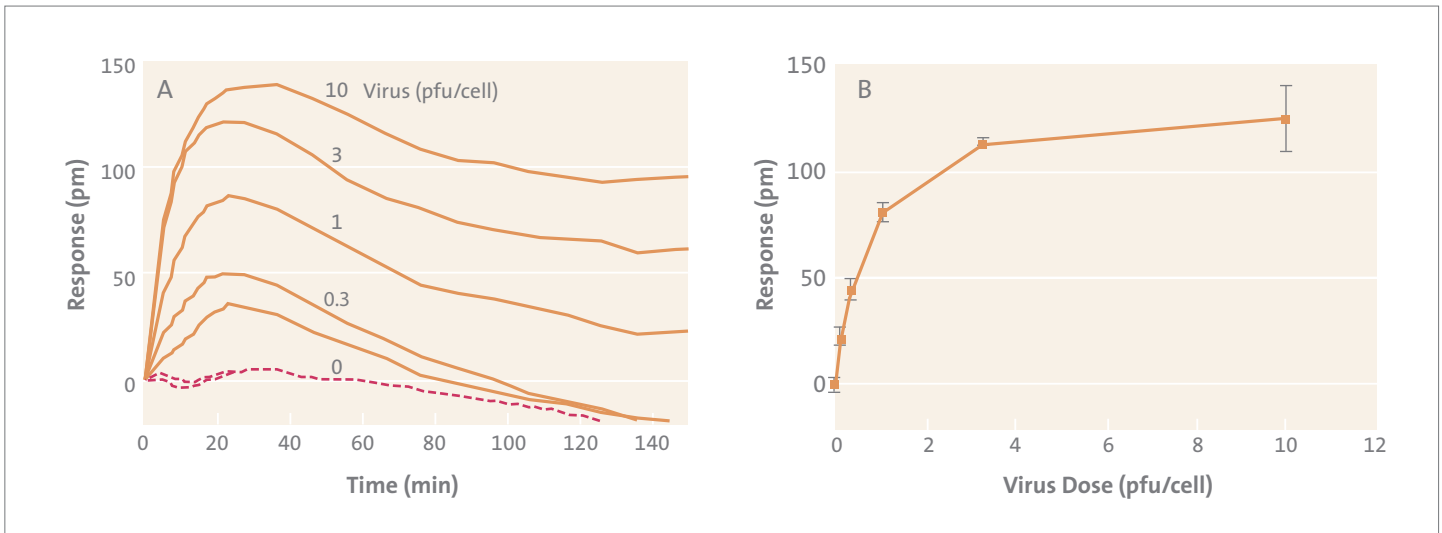
The late phase response was characterized by a gradual decrease in the Epic signal with time. The higher the HRV dose, the earlier the decrease in the Epic signal.  $T_m$ , defined as the time at which half of the maximal signal is achieved, shifts to the left with increasing virus dosages (Figure 4). Both HRV14 and HRV16 showed similar patterns with only the result from HRV14 shown in the figure.



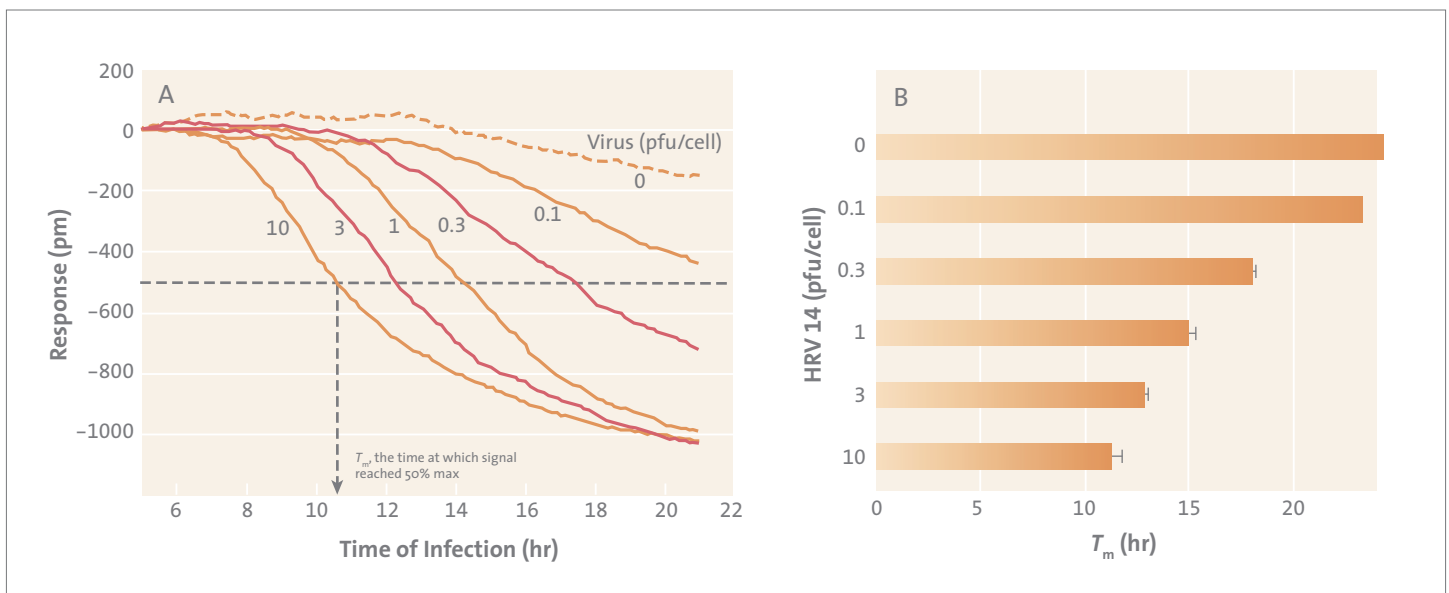
**Figure 1.** Schematic of Corning Epic assay which involves cell seeding, virus addition and reading.



**Figure 2.** HeLa cell response to HRV14 infection. The time trace is characterized by an early stage (0 to 2 hr), a middle quiet stage (2 to 8 hr) and a late stage (10 to 24 hr) responses. Culture medium was used as a negative (no virus) control (dotted line).



**Figure 3.** Early stage response of HeLa cells to HRV14 infection. The Corning Epic signal increased rapidly in the first 20 minutes post virus addition and then decreased gradually in the next two hours (A). The signal magnitude was virus dose-dependent indicating that the signal was triggered by virus infection (B).



**Figure 4.** Late stage response of HeLa cells to HRV14 infection. The Corning Epic signal decreased with time and reached plateau after 22-hour infection (A).  $T_m$  was shown for each virus dose used (B).

To compare a Corning® Epic® assay with a traditional CPE assay, cells grown and infected with 10 pfu/cell HRV14, the highest dose used in an Epic assay with either an Epic microplate or 384 well tissue culture microplate, were assayed with the CPE method. Surprisingly, no significant effect was detected in the CPE assay within 24 hours,

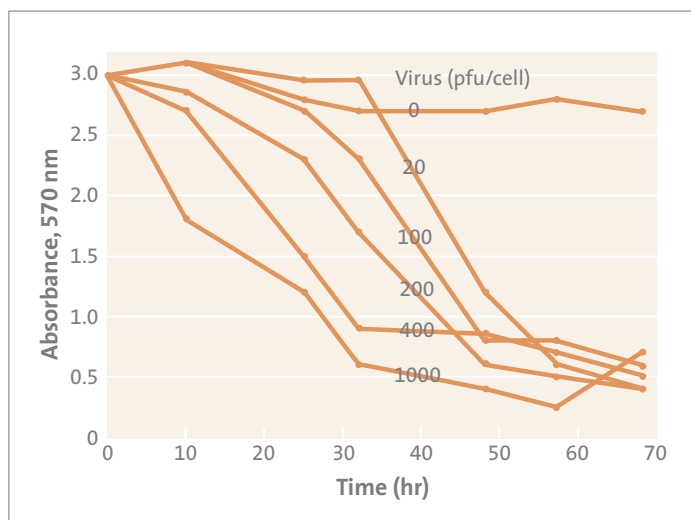
whereas the Epic assay had completed. CPE assays with higher virus doses and longer incubation times were conducted with 96 well tissue culture microplates. As shown in Figure 5, at 20 pfu/cell (corresponding to 2 ng/cell), a CPE effect could not be detected until 30 hours post infection. A higher virus dose led to earlier detection of CPE effect.

## Conclusions

- ▶ The Corning® Epic® System can detect the effect of virus at as low as 0.1 pfu/cell.
- ▶ CPE assays require 100 times more virus for the viral activity to be detected within 24 hours.
- ▶ At 10 pfu/cell, the Epic System can detect the virus effect in as few as 10 hours, while CPE requires at least 30 hours.
- ▶ Epic assay requires little handling of viral contaminated reagents and supplies.

## References

1. Fang, Y (2006) *Assay Drug Dev Technol* 4(5):583-595.
2. Hadfield AT, Lee W, Zhao R, Oliveira MA, Minor I, Rueckert RR, and Rossmann MG (1997) *Structure* 5(3):427-441.
3. Rueckert RR, and Pallansch MA (1981) *Methods Enzymol.* 78:315-325.



**Figure 5.** CPE assay of HRV14 infection. Cells were grown in a 96 well tissue culture plates overnight. The virus was then added to the wells and incubated at 35°C. At the time points specified, cells were stained with crystal violet and the absorbance at 570 nm was measured according to Material and Methods. 0 to 1000 pfu/cell were used.

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